α-ALKOXIMINO ACIDS AND THEIR AMIDES

By

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TABLE OF CONTENTS

INTRODUCTION

The complex structure of proteins found in nature has long been a real barrier to investigators in the field of protein chemistry and has impeded their learning the reasons for the varying properties often encountered with these involved molecules. The very early workers discovered that when proteins were subjected to the action of acids, alkalies and certain enzymes (themselves partially protein), a breakdown in the molecule occurred with the production of α-amino acids predominantly. To date, some twenty to twenty-five such acids have been identified from these hydrolysates. The manner in which these acids are linked together is no longer a mystery. Several investigators (1, 2) have noted that on enzymatic hydrolysis of proteins with trypsin or pepsin that amino and carboxyl groups are liberated in the ratio 1:1. This suggests the amide linkage and is supported by the fact that few free amino and carboxyl groups are found in proteins (3). The free amino groups occurring are largely due to the diamino acids, such as lysine, and only a very small fraction is due to the monoamino acids. Another supporting bit of evidence for the amide linkage is that synthetic peptides when subjected to the action of enzymes hydrolysing naturally occurring proteins, are aplit at

the amide linkage. Furthermore, this linkage has been found in simple substances occurring in nature such as pantothenic acid, glutathione and hippuric acid. Most of the simpler peptides isolated from hydrolysates have been synthesized, which in itself constitutes partial synthesis of the protein molecule. Consequently the protein molecule may be rather definitely said to be made up of a great number of these amide linkages between the α-amino acid and the carboxyl group of its neighbor. This linkage is classically referred to as the peptide linkage.

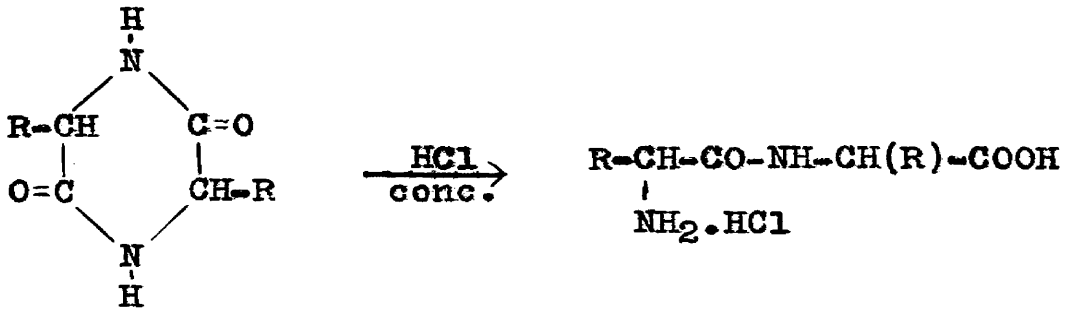
Unfortunately, the investigator is this field is hampered since hydrolytic procedures do not reveal the order of the amino acids as they occurred in the protein. Some simpler peptides, however, have been identified (4, 5). Synge (6) has given a very good review on the subject of protein hydrolysates which well summarizes the progress made in this difficult study. However, the surest way to learn the secret of the protein is to prepare the simple molecules bearing the peptide linkage and build up to the larger molecules. In this way the exact order of the amino acids may be established. This avenue is not one that has yielded immediate success since the physiological mechanism of protein action has not been explained through synthetic procedures. However, the possibility of discovering this source of activity must certainly not be excluded and in all probability the

answer to the problem is along this route.

The first claim for the synthesis of the peptide linkage was made by Curtius (7). However, all his efforts to hydrolyse selectively benzoylglycylglycine did not give him the desired peptide, glycylglycine. In 1901, Emil Fischer (8) succeeded in preparing the first peptide through the hydrolysis of 2,5-diketopiperazine with concentrated hydrochloric acid, obtaining glycylglycine hydrochloride. The free peptide was obtained as a crystalline solid by preparing the silver salt and treating it with hydrogen sulfide.

Since this successful endeavor, a number of procedures have been evolved which have been successful to different degrees. The methods that have been utilized are set forth briefly as follows:

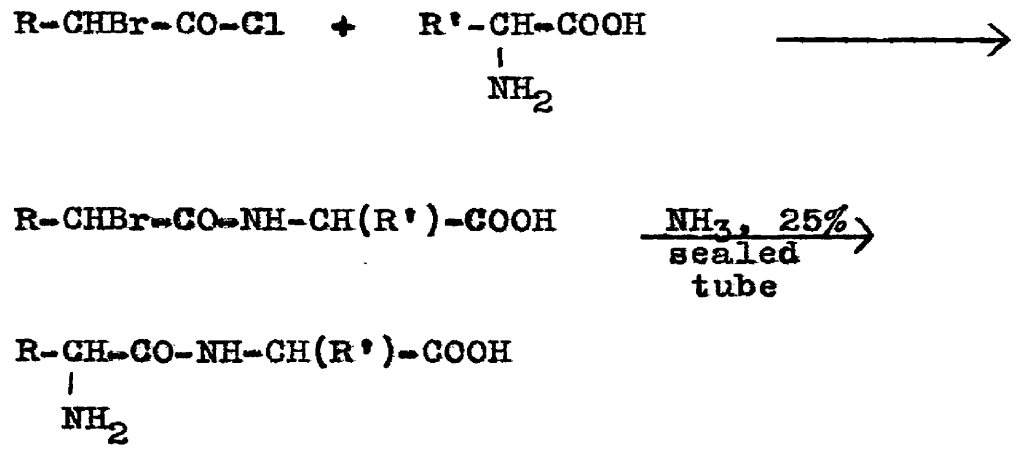
1. Hydrolysis of diketopiperazines



This procedure enjoys limited use since it is necessarily restricted to the synthesis of dipeptides. Another limiting feature is the availability of the diketopiperazine intermediate. If an unsymmetrical anhydride were used, two peptides would be obtained and the difficulty in separating two such isomerides would be nearly

insurmountable. The most complicated peptide synthesized through this method was l-cystinyl-l-cystine (9).

2. Halogenacyl method



This is Fischer's (10) classical method for the synthesis of peptides. Realizing the necessity for extension of peptide syntheses to a chain of more than two amino acids, he first applied it in the preparation of diglycylglycine. Yields are usually improved by the use of the amino acid or peptide ester, rather than the free acid (11). The largest peptide made by Fischer, using this method partially, was l-leucyltriglycyl-l-leucyltriglycyl-l-leucyloctaglycylglycine, an octadecapeptide (12). The largest peptide ever prepared was made in 1916 by Abderhalden and Fodor (13) who succeeded in making a nineteen membered compound.

One novel method used for peptide esters for the replacement of the halogen by the amino group involves substitution of the halogen by the azido group and then reducing with platinum to the amino group (14, 15). Hydrolysis yields the peptide.

There are a number of limitations to this classical procedure however. Hydroxyamino and diamino acids can be used in dipeptide work, but the series can not be extended because phosphorus pentachloride does not form the dipeptide acid chloride. In addition, such peptides as phenylalanylglycine can be prepared in low yields only, because ammonia splits out halogen hydride yielding a cinnamamide (16), in the following fashion.

$$C_6H_5\text{-}CH_2\text{-}CHBr\text{-}CO\text{-}NH\text{-}CH_2\text{-}COOH \xrightarrow{NH_3}$$

$$C_6H_5\text{-}CH{=}CH\text{-}CO\text{-}NH\text{-}CH_2\text{-}COOH$$

3. Ester condensation

Fischer observed that esters of amino acids possessing a β- or ε-amino group condensed to give dipeptides (17).

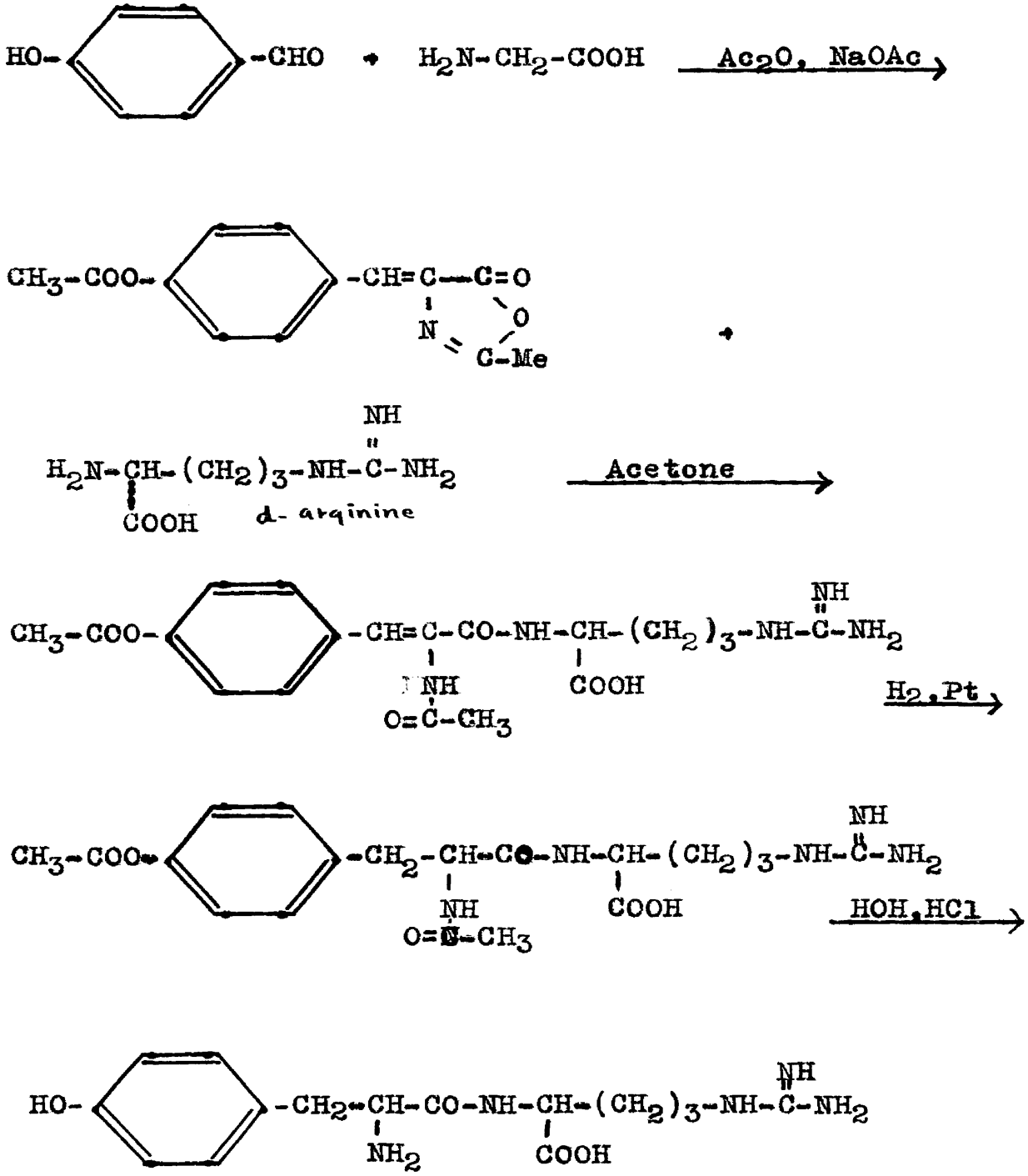
$$H_2N\text{-}(CH_2)_n\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOMe \xrightarrow{100^\circ}$$

$$H_2N\text{-}(CH_2)_n\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}CO\text{-}NH\text{-}(CH_2)_n\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}COOMe$$

Similarly, di- and tripeptides can be condensed in good yield (better than 70% in some cases) to yield tetra- and hexapeptide esters (18, 19). The usefulness of this reaction is limited to extension of the peptide chain, since most esters condense to give diketopiperazines. The three methods given thus far are all a result of Fischer's attack

on the structure of peptides. He summarized this work very beautifully up to 1906 in a very long paper to the German Society (20).

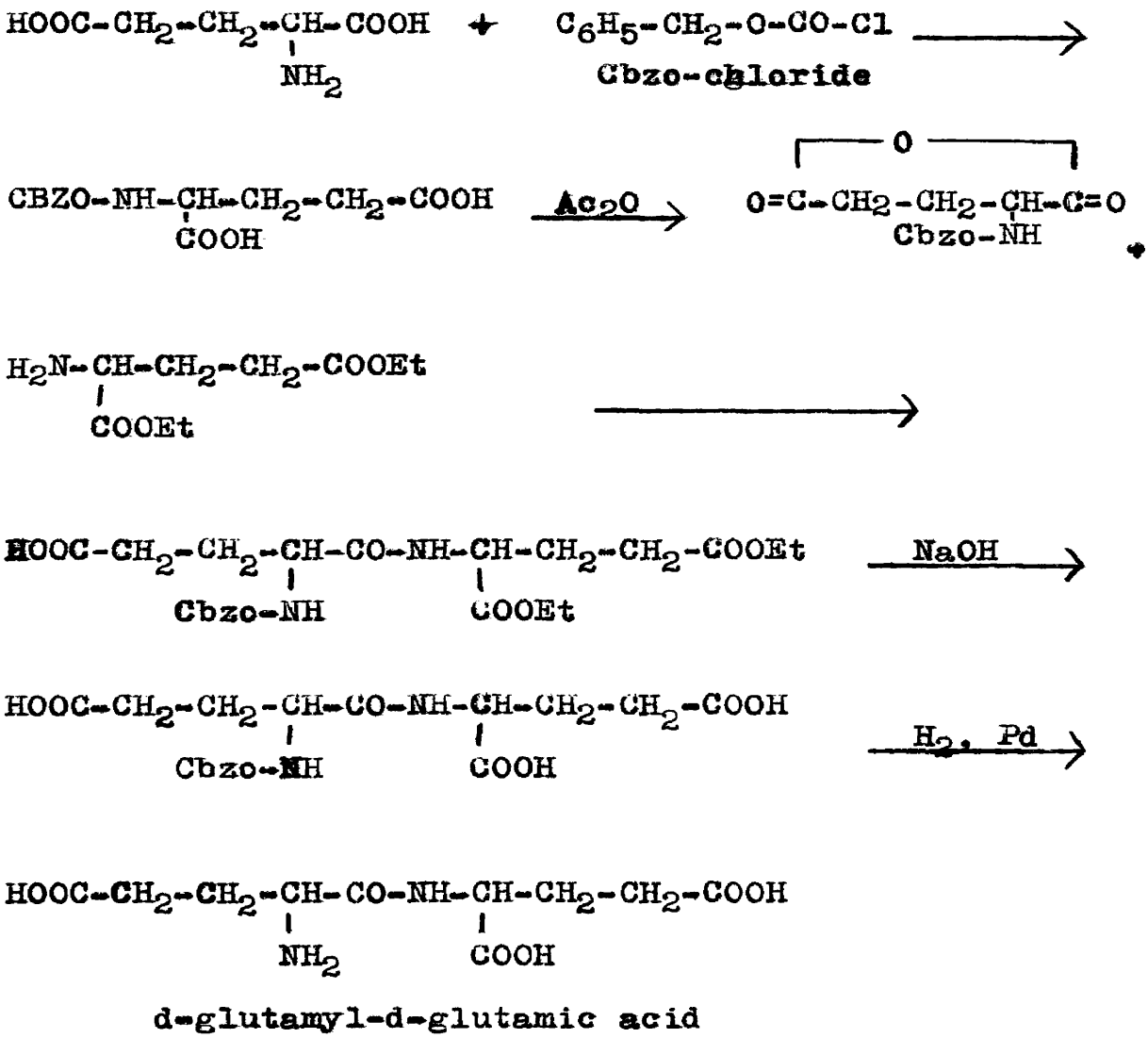
4. Azlactone synthesis



d-tyrosyl-d-arginine

It was not until 1926 that the azlactone method of Bergmann was described (21). Erlenmeyer (22) first described the formation of oxazolones (azlactones) in 1895 and later used them for preparing ∝-amino acids (23). The beauty of the azlactone method for peptide preparation is that for the first time the more complicated amino acids could be used. Unfortunately, the yields are not good in the procedure and the l-peptides are difficultly accessible.

5. Carbobenzyloxy method

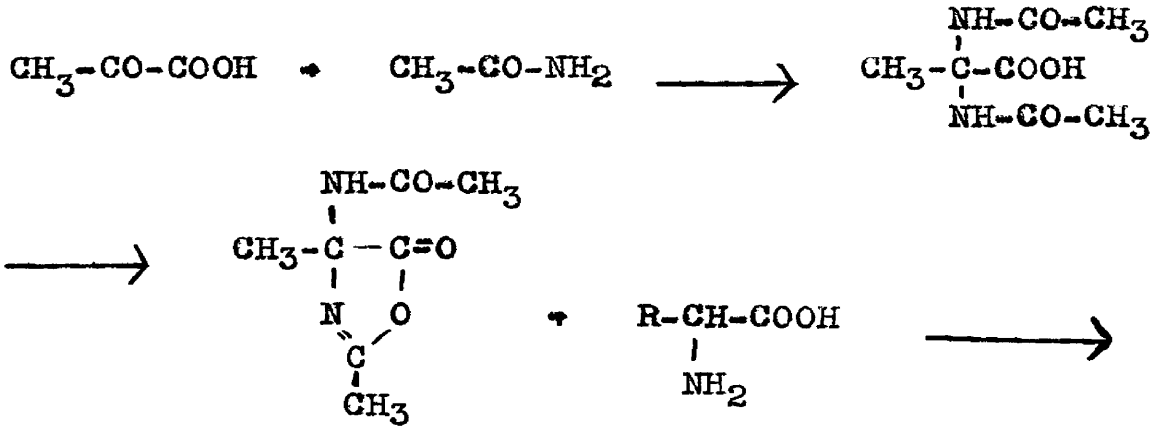


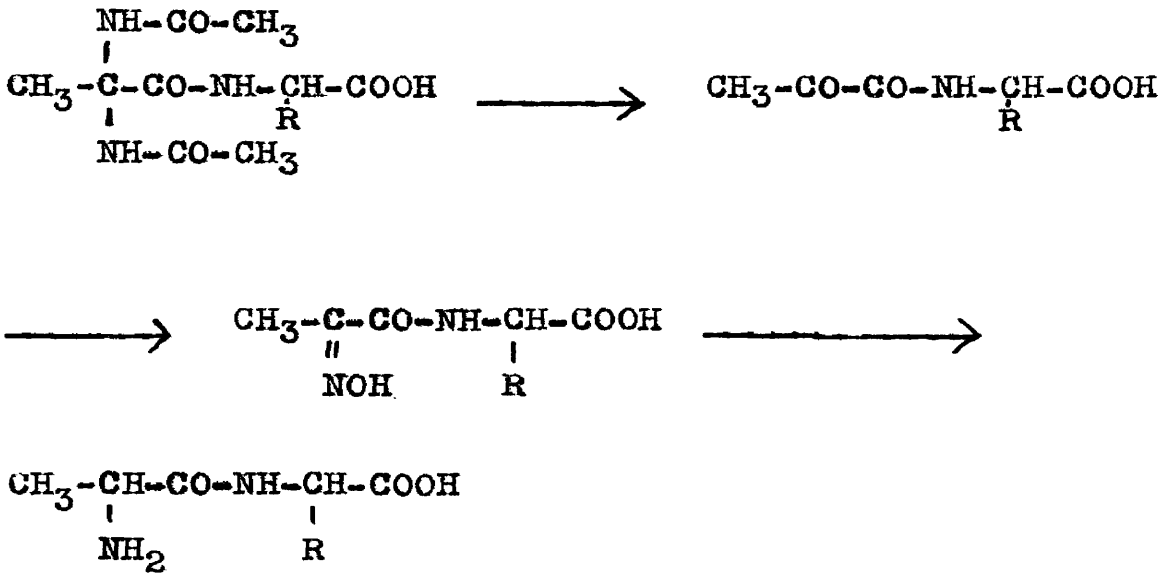
d-glutamyl-d-glutamic acid

Thirty years after Fischer synthesized glycylglycine, Bergmann and Zervas (24) prepared d-glutamyl-d-glutamic acid in 31% overall yield. This synthesis is without doubt the most elegant of all peptide syntheses known today. It is interesting that Fischer (25, 26) had used carboethoxy and carbomethoxy chlorides to block the amino and hydroxyl groups of several acids, but had been unable to remove them with any great success. The important step in Bergmann's synthesis was the catalytic hydrogenolysis of the benzyl group.

By using other methods to remove the carbobenzyloxy group, the sulfur containing peptides have been obtained, notably glutathione (27). A rather comprehensive review of this method has been made recently by Bergmann and Fruton (28). The best feature of this synthesis is that optically active amino acids can be used without affecting their configuration. The peptides thus obtained are excellent for hydrolytic studies involving enzymes.

6. <u>α-Keto acid method</u>





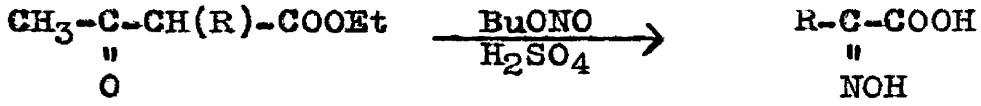
Using this method, Schemin and Herbst (29) were able to prepare several alanyl peptides. For the preparation of the pyruvyl derivatives, the method of Bergmann and Grafe (30) was used. In the reduction to alanylphenylalanine, the aromatic ring was reduced and only the cyclohexyl derivative could be made. This method is further stymied by the difficulty encountered in the preparation of the pyruvyl derivatives.

Although the procedures outlined are all a step forward, none could be considered ideal. Undoubtedly, the most important procedure is the carbobenzyloxy method since it allows the synthesis of complex peptides and does not interfere with optical activity. This method and the halogenacyl method also leave no doubt as to the sequence of components in the peptide.

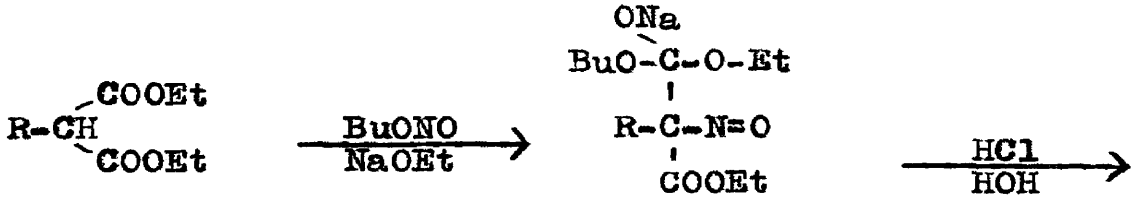
However, the Bergmann method requires the free amino acid for the synthesis. In the case of certain amino acids this is a problem, but not necessarily a drawback. Nevertheless, the use of a method which incorporates the synthesis

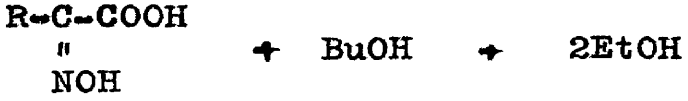
of the amino acid in situ, when the peptide is formed, would certainly be helpful and possibly more economical in special cases. Fischer's classical method, of course, has this as one of its features.

Hamlin (31) demonstrated that oximino acids could be prepared in high yields from substituted acetoacetic esters using a modification of Bouveault and Locquin's nitrosation procedures (32, 33, 34).



This method is limited, however, to compounds in which R is an alkyl or unsubstituted benzyl group. Barry (35) subsequently developed a method of more general application for synthesizing oximino acids, which is also useful if the aromatic nucleus is highly substituted. Using an alkyl nitrite in the presence of sodium ethoxide, he was able to obtain oximino acids in yields ranging from 60 to 75% from the substituted malonic and acetoacetic esters.





He obtained even better yields when the substituted malonic acid and alkyl nitrite were allowed to react in the presence of hydrogen chloride. Mattocks (36) has shown that such oximes as β-(3,4-diethoxyphenyl)-α-oximino-propionic acid and its methylene dioxy analogs are readily accessible through these methods.

The success of these workers lays the foundation for the amino acid synthesis and subsequently peptides. Hamlin, using Hartung's reduction (37) with palladium chloride on charcoal in the presence of hydrochloric acid, was able to obtain a number of amino acids in good yield.

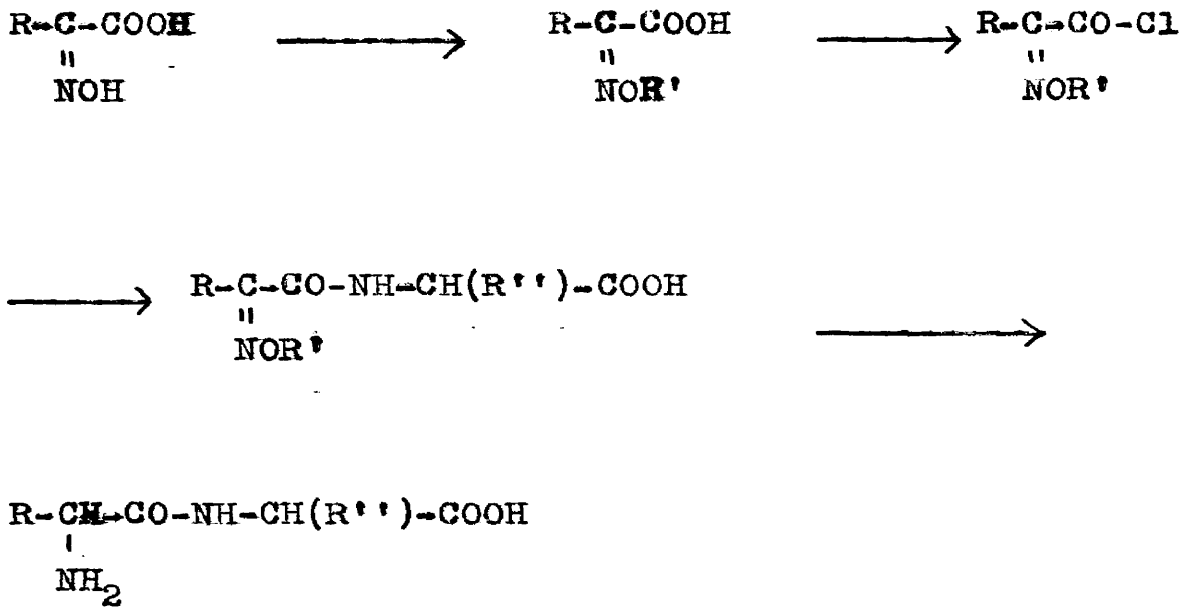
$$\underset{\underset{\text{NOH}}{\|}}{\text{R-C-COOH}} \xrightarrow[\text{HCl}]{\text{H}_2,\ \text{Pd}} \underset{\underset{\text{NH}_2}{|}}{\text{R-CH-COOH}}$$

Mattocks has also used this reduction for the preparation of the amino acids more highly substituted in the aromatic nucleus. Since amino acids are rather readily available by this method, it is feasible that the ideal intermediate for the synthesis of peptides might be through the oximino acids.

Waters (38), using a great variety of unsuccessful procedures, demonstrated that it was impossible to obtain the keto acids from the oximino acids. Moreover, it was shown to be impossible to prepare the oximino acid chlorides using the conventional reagents, because of the reactivity of the oximino group. Hence, two rather convenient routes

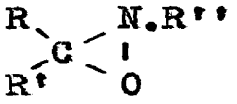
through the oximino acids were barred.

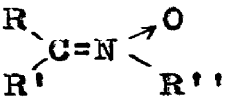
Consequently, the obvious procedure was to block the oximino group. Waters found that this may be done by preparing the O-ethers, R-C(=NOR)-COOH. These compounds are typical carboxylic acids from which the acid chlorides may be obtained. These acid chlorides react with amines to form the corresponding amides. E.g.,



Oxime ethers are not new, especially the lower alkyl derivatives. They were usually synthesized by treating the oxime in sodium ethoxide with the appropriate alkyl iodide. It was through this method that the first ketoxime ether of an aromatic ketone was prepared (39). Unfortunately, the directions are not as clear as they should be. In 1889, Beckmann (40) recognized that he had obtained two distinct compounds on the benzylation of benzaldoxime.

Shortly afterwards (41), Goldschmidt showed that both a nitrogen and oxygen alkyl derivative were obtained when aromatic oximes were reacted with alkyl iodides. Dunstan and Goulding (42) in 1901 gave an excellent review of the structure of these two types of oxime ethers and showed that invariably, the N ethers, along with the O ethers, were obtained when oximes were alkylated with iodides. The early workers in this field assigned the cyclic structure (I) to these nitrogen ethers. This structure

(I) 

(II) 

has been supplanted more recently by the so-called nitrone structure (II). The physical properties of the nitrogen ethers have shown this to be most true. The position in regard to these isomers of oxime ethers has been well summarized by Taylor and Baker (43).

As will be pointed out later, the demonstrated ease with which benzyl ethers can be catalytically split makes this alkyl group of particular importance, for if the oxime group were blocked with the benzyl group, it would be expected that the ether could be split more easily than if other alkyl groups were used. These ethers are not new, the benzyl ether of oximinomalonic ester being reported in 1881 by Conrad and Bischoff (44). These workers prepared the ether from the oximino ester in alcoholic sodium ethoxide using benzyl chloride as the benzylating agent. The

benzyl ether of acetone oxime has been prepared similarly by Meyer (45) and Janny (46). Janny established, by hydrolysis with hydriodic acid, that the benzyl group was bound to the oxygen rather than the nitrogen. Spiegler was the first to study the oxime ethers of the aromatic compounds. Like previous investigators with aliphatic oximes, his alkylations were carried out in alcoholic sodium ethoxide. Brady and Klein (48),using similar techniques, were able to prepare the p-nitrobenzyl ether of benzaldoxime in 89% yield. This ether was especially suitable in the study of the geometrical isomers obtained when oximes were alkylated.

Hantzsch (49) prepared some interesting oxime ethers of α-oximino acetic and propionic acids. His procedure for the preparation of the oxime was to carry out the alkylation of the oximino acid with chloroacetic acid by heating in aqueous potassium hydroxide for six to eight hours. All of Hantzsch's products were shown to be O-alkyl derivatives.

Very little is known about the catalytic dealkylation of oxime ethers. Jones and Major (50) have studied the reduction of certain O-alkyl derivatives of oximes. Using platinum oxide in dilute ethanol with hydrochloric acid, they obtained primary and secondary amines, along with unrecovered oxime ether. Adkins and Reeve (51) were able to prepare the ethyl ester of threonine from ethyl O-ethyloximinoacetoacetate using Raney nickel at 90° and with 300

atmospheres of hydrogen.

The catalytic debenzylation of O and N bound benzyl and benzal groups, however, is better known. Rosenmund (52) first showed that benzyl benzoate in xylene was split to toluene and benzoic acid, with palladium on barium sulfate. Later, he extended the work to the reduction of mandeloacetates (53). The same palladium catalyst was used by Kariyone and Kimura (54) in a study of the reduction of various acetals of benzaldehyde. The method as reported by these workers was excellent. Wolfes and Krauss (55) cited, in an often quoted German patent, the use of palladium for the reductive debenzylation of ethers.

Freudenberg and co-workers (56, 57) have shown that certain benzyl and benzal sugars can be split using platinum black. For benzyl-diacetone-glucose, they show that hydrogenation in ethanol does not yield the desired product, while acetic acid is satisfactory. The same situation does not pertain to benzal (α-methyl) glucoside, however. Good yields with benzyl sugars were also obtained with sodium and alcohol. Sigmund (58) was able to show that platinum was excellent for certain acetals but that the catalyst produced methylcyclohexane.

Catalytic methods, particularly with palladium, have been widely used for the removal of benzylidene residues for sugars (59, 60). Like Freudenberg, Fischer (61, 62) debenzylated several benzyl derivatives of sugars with with palladium in acetic acid. This was the reduction employed by Bergmann and Zervas (24) in their classical

peptide synthesis, except that methanol was used in conjunction with the acetic acid. DuVigneaud (27) removed the carbobenzyloxy group with sodium in liquid ammonia and Harington and Mead (63) used phosphonium iodide in preparing optically active glutathione through the Bergmann and Zervas method.

More recently Baltzly and Buck (64) studied the hydrogenolysis of O- and N- bound benzyl groups. They found that substituents on the α-carbon atom and on the benzene ring stabilized the system towards palladium. Simonoff (65) carried out debenzylations using palladium catalysts and gives a review of the catalysts used for debenzylation. Mattocks (66) has combined the use of platinum and palladium in the quantitative debenzylation of benzyl β-alanine.

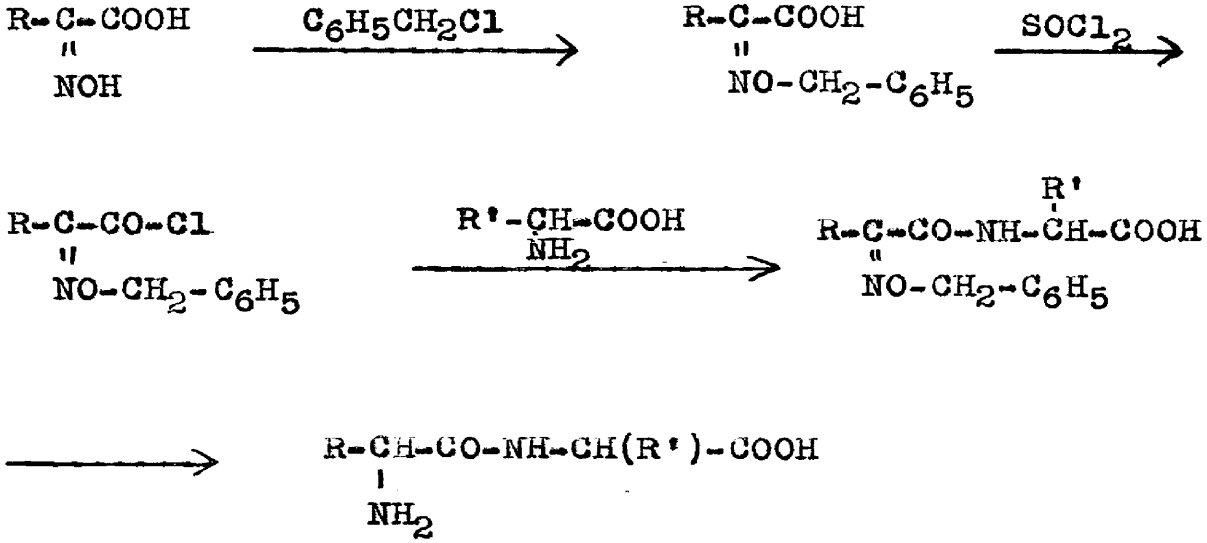
Not too much work seems to have been done with Raney nickel as a debenzylating agent. However, Van Duzee and Adkins (67) in a rather good survey of hydrogenolysis of ethers in general, showed that benzyl ethers are definitely easier to hydrogenolyse than other ethers, the reaction being carried out in ether at temperatures ranging somewhat over 100°. They attribute this ease to the more reactive β-double bond found in benzyl ethers.

Waters in a few preliminary experiments with the alkoximino acids, was led to believe that the benzyl derivatives were much easier to reduce than the ethoximino compounds. Raney nickel was not successful. As will be shown in the experimental section, the debenzylation itself is

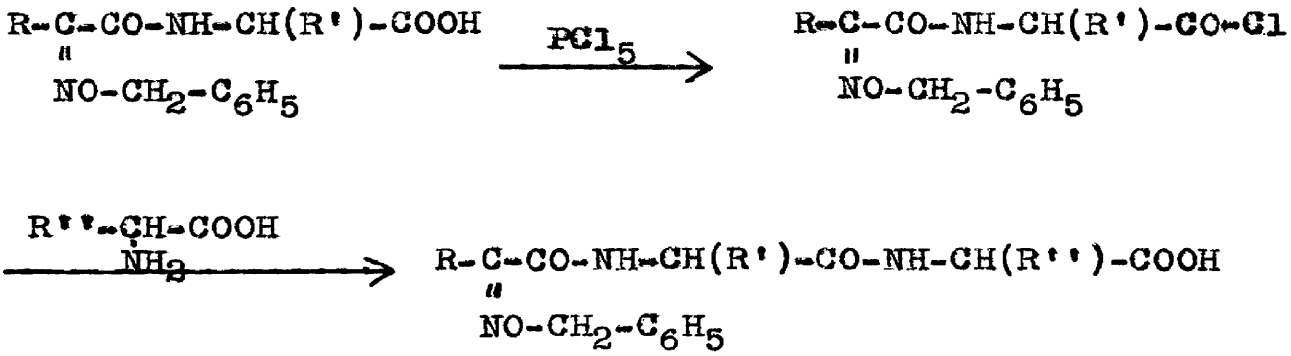
not too difficult, apparently, but the reduction to the peptide is somewhat subborn.

RESEARCH AIM

Previous work in these laboratories gave promise that peptides might be satisfactorily prepared according to the following scheme:



Since dipeptides are but the first step in a really successful synthesis for peptides, an effort has been made to extend the work to peptides having more than two amino acid constituents, thus:



The next logical step, of course, would then be hydrogenation

to the tripeptide. In such a way the various amino acid constituents could be tied together in a known sequence. This is one necessary prerequisite for a study of peptides and their reactions.

Waters's work was limited to the preparation of several α-ethoximino acids and two α-benzyloximino acids with the corresponding acid chlorides and anilides. In the first stages of the investigation reported here, studies were made of the benzylation of oximino acids with the aim of making the synthesis more practicable. Furthermore, the extension of Waters's work to the establishment of the peptide linkage and the reductions of the intermediate amides had to investigated.

This dissertation describes:

a. The synthesis of intermediates necessary for the initial benzylation studies.

b. The benzylation of α-oximino acids under varied conditions.

c. The preparation of the acid chlorides of the α-benzyloximino acids.

d. The establishment of the peptide linkage with various amino acids.

e. The reduction of these α-benzyloximino acid amides.

f. The attempts to extend the synthesis beyond two amino acids.

EXPERIMENTAL SECTION

I. INTERMEDIATES

A number of the intermediates used in this work were purchased from various commercial sources and used without purification. Among these were the following "white label" products from Eastman Kodak Co.: ethyl ethylmalonate, ethyl n-butylmalonate, isopropyl bromide, glycine, dl-alanine, l(-)leucine, dl-leucine and l(-)tryptophane. The ethyl α-oxalpropionate used was obtained from the U. S. Industrial Chemicals Co. The sec-butyl and isobutyl bromides from the Columbia Organic Chemicals Co. l(+)Glutamic acid was obtained from the Pfanstiehl Chemical Co. and dl-phenylalanine from A. D. McKay.

Some of the chemicals used were redistilled before use, such as ethyl malonate, ethyl acetoacetate and thionyl chloride from Eastman Kodak Co.; C. P. grade benzyl chloride, usually slightly yellow in color as obtained from the J. T. Baker Chemical Co.; "white label" butyl nitrite from Eastman was found to be impure. The three compounds last mentioned were all stored in the refrigerator after purification.

A. Malonic Esters and Acetoacetic Esters.

<u>Ethyl benzylmalonate</u>

The usual preparation of this compound has been described in Organic Syntheses (68). A slight modification of this method, consisting of raising the molar quantity of

benzyl chloride, gave better yields.

In a 2-liter, 3-necked flask, equipped with a Hershberg stirrer, dropping funnel and a condenser, provided with a drying tube, was placed 1 liter of absolute ethanol (distilled from calcium oxide). To this was added 46 gm. (2 moles) of sodium at such a rate as to keep the alcohol refluxing (1 to 1½ hours). Then 480 gm. (3 moles) of ethyl malonate was added all at once through the funnel, followed by 253 gm. (2 moles) of benzyl chloride, which was added dropwise over a period of 2 hours. The reaction mixture was refluxed from 20 to 24 hours and about 800 ml. of alcohol removed; the residue was poured into 750 ml. of water and allowed to separate in a separatory funnel. After drawing off the dark red product, the aqueous layer was extracted twice with ether-benzene and the extract added to the product. On distilling through an 8-inch Vigreux column, the excess malonic ester (b. p. 90-91°/15 mm.) was recovered and 328 to 331 gm. of ethyl benzylmalonate (b. p. 117-118°/.25 mm.) was obtained, representing a 66 to 67% yield. A sizable amount of liquid residue remained, presumably ethyl dibenzylmalonate.

When the alcohol was treated with sodium and distilled from ethyl phthalate, according to the method of Manske (69), the yield was boosted to 73% of theory.

Ethyl sec-butylmalonate

This compound was prepared in 83% yield using the method described by Organic Syntheses (70). This procedure is essentially the same as that described for ethyl

benzylmalonate. It is necessary to use a rather good column to separate the ethyl malonate that distils over, since the product boils at 105-109°/10-11 mm.

Ethyl isobutylmalonate

This compound was prepared by the above procedure in 81% yield. The product boiled at 98-108°/10-11 mm.

Ethyl isopropylmalonate

The preparation of this compound was carried out according to the method of Adams and Kamm (71), which is much like the above syntheses. A yield of 60% of theory was obtained; the product boiled at 112°/28 mm.

Ethyl methylmalonate

This compound was most conveniently prepared through ethyl α-oxalpropionate according to the method of Cox and McElvain (72).

Three hundred three gm. (1.5 moles) of ethyl α-oxalpropionate was heated in a 1-liter, round-bottomed flask, equipped with a condenser, on an oil bath for eight hours at 150-160°. When the evolution of carbon monoxide ceased, the liquid was distilled by elevating the bath temperature to 220°. A colorless liquid, 238 gm. (91% of theory), was obtained boiling at 194-198°.

Ethyl sec-butylacetoacetate

All attempts to prepare this compound by standard procedure (73) were discouraging in that the yields of desired product were only 15 to 20% of theory. This was also found to be true for the isomeric isobutyl compound.

Varying the quantity of halide and acetoacetic ester had no effect on the end result. Substitution of isopropyl alcohol for ethyl alcohol was no advantage. This alcohol reacts much slower than ethyl alcohol with sodium and, furthermore, sodium isopropoxide is not nearly as soluble in isopropyl alcohol as sodium ethoxide is in ethyl alcohol. In the case of ethyl isobutylacetoacetate, on at least one occasion, when the reaction mixture was refluxed somewhat longer than usual (20 hours), a liquid boiling at 60°/13 mm. was obtained. This boiling point is much too low for either the product or unreacted acetoacetic ester. Ammonolysis with concentrated ammonium hydroxide at room temperature for one week yielded a white solid melting at 118-118.5°. This corresponds to the amide of isocaproic acid which melts at 120-121° (74). The liquid product was probably ethyl isocaproate. Recent work (75, 76) has shown that secondary halides and halides bearing substituents on the alpha carbon atom always give low yields in the acetoacetic ester condensation. Fortunately, for the purposes of this work, the corresponding malonic esters can be obtained in good yields.

B. Oximino acids.

The oximino acids used in this work were prepared by the alkaline nitrosation of substituted malonic esters, as reported by Barry (35). The yields are not changed markedly if the quantity of butyl nitrite is varied between one-third and one mole of excess nitrite. It does not seem

to be necessary to concentrate the reaction mixture to a pasty mass, especially if the butyl nitrite is of good quality. The following example demonstrates the general procedure used for the preparation of the oximino acids used in this work.

β-Phenyl-α-oximinopropionic acid.

Eleven and one-half gm. (.5 mole) of sodium was added to 1 liter of absolute alcohol contained in a 2-liter, 3-necked, round-bottomed flask equipped with a condenser, drying tube, stirrer and dropping funnel. After reaction of the sodium, 125 gm. (.5 mole) of ethyl benzylmalonate was added to the hot mixture, which was then cooled in an ice-salt bath to 0°. Then 103 gm. (1 mole) of butyl nitrite was added slowly beneath the surface of the mixture over a period of about one hour keeping the temperature below 5°. The cooling bath was removed, the mixture allowed to reach room temperature slowly (one hour) and then heated to reflux. Suction was applied to remove the butanol and ethanol until a volume of about 200 ml. remained. Then 600 ml. of ice and water were added, the product acidified with hydrochloric acid and extracted with ether. The ether extract was extracted with 10% sodium hydroxide, which was then heated on the steam bath for about an hour. After adding ice, the mixture was acidified with concentrated hydrochloric acid. The precipitate which formed was then filtered and dried in vacuo over phosphorus pentoxide. Yield: 95% of light tan solid melting at 160°. Barry reported 169° for the recrystallized product.

The oximino acids obtained by this method usually melted 10 to 20° below the reported values and were tan in color. The lower oximes, such as α-oximinopropionic acid are more soluble in water. Consequently, the mother liquor remaining after filtering off the initial precipitate was extracted with ether in a continuous extraction apparatus for 24 to 48 hours. The ether was then blown off with air. For the lower acids the amount of product obtained by this extraction was as much as one-fifth the total yield and decreased progressively as the molecular weight increased.

α-Oximinopropionic acid.

This acid was prepared like the above compound. The yield of crude product (m. p. 163°d.) ranged from 62 to 70% of theoretical. Inglis and Knight (77) reported a melting point of 180-181° for the pure material.

α-Oximinobutyric acid.

Using the same procedure, a yield of crude material (m. p. 131°d.) representing 66% of theoretical was obtained. Barry (35) reported 154° for the pure product.

α-Oximinohexanoic acid.

Using one-third mole of excess butyl nitrite, an 84% yield was obtained. The reddish-brown, crude solid melted at 120°d. Barry (35) found the pure product melted at 135°.

α-Oximinoisocaproic acid.

This compound was prepared in 54 to 60% yields using the general procedure given. The crude material melted at 147°d., but on recrystallization from ligroin, the white needles melted at 153-154°. Locquin (34) reported a melting

point of 159-160°.

β-Methyl- α -oximinobutyric acid.

A poor yield of this compound was obtained on one run. The experiment was not repeated and the reason for the poor yield was not determined. The light-tan, crude product melted at 148°d.; Bouveault and Wahl (78) reported 163-165° for the pure product.

β-Methyl-α-oximinovaleric acid.

Repeated runs with this compound gave yields of only 22 to 33%. The crude material melted at 140°d., but on recrystallization from ligroin, the white needles melted at 152-153°. Hamlin (31) reported a melting point of 145°d.; Bouveault and Locquin reported 164°. Whether this method is not applicable to this and the above ester was not determined.

Since the products obtained by this method were usually of fair purity, they were often used without subsequent recrystallization for the preparation of the benzyl ethers.

C. Butyl nitrite.

The method of Noyes (80) as modified by Barry was used in the preparation of this compound.

$$2\ C_4H_9OH + 2\ NaONO + H_2SO_4 \longrightarrow 2\ C_4H_9ONO + Na_2SO_4 + 2\ HOH$$

In a 3-liter, 3-necked, round-bottomed flask, equipped with a Hershberg stirrer, thermometer and a dropping funnel with the stem leading beneath the surface,

was placed a solution prepared from 500 ml. of water and 380 gm. (5.5 moles) of sodium nitrite. To it was added a kg. of finely crushed ice. A previously chilled mixture of 100 gm. of ice, 136 ml. of concentrated sulfuric acid and 457 ml. (5 moles) of butyl alcohol was then added slowly over a period of one hour. The flask was cooled in an ice-salt bath during this time, and the temperature was not allowed to rise over 6°. After the addition was completed, the mixture was allowed to separate and water was added to dissolve the salt in the flask. The butyl nitrite layer was separated in a 2-liter separatory funnel and washed with two 50 ml. portions of a solution of 2 gm. of sodium bicarbonate and 25 gm. of sodium chloride in 100 ml. of water. The light-yellow liquid obtained (453 gm., 88% yield) was stored in the refrigerator over anhydrous magnesium sulfate. The material was sufficiently pure to use directly, without distillation, in the nitrosation of the malonic esters.

II. BENZYLATION OF OXIMINO ACIDS

Waters was able to prepare certain α-ethoximino acids in rather good yields. However, the preparation of the corresponding α-benzyloximino acids was not attended with similar success. He was able to prepare β-phenyl-α-benzyloximinopropionic acid in 56% yield and α-benzyloximinohexanoic acid in 42% yield. These benzylations were

carried out in the presence of aqueous sodium hydroxide and acetone. Unfortunately, the difficulties encountered in the isolation of the benzyl derivatives accounted for the lowered yields reported. Consequently, it was considered advisable to study the benzylations; e. q., the effect of temperature, ratio of reagents and to improve the method of isolation of the product. This study was made with β-phenyl-α-oximinopropionic acid. Waters's found that the sodium salt of this acid is soluble in the benzyl alcohol formed during the reaction and the isolation of the pure acid from the salt is difficult. The work repeated here bears this out.

$$C_6H_5\text{-}CH_2\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}COOH \quad + \quad C_6H_5\text{-}CH_2Cl \xrightarrow{NaOH}$$

$$C_6H_5\text{-}CH_2\text{-}\underset{\underset{NO\text{-}CH_2\text{-}C_6H_5}{\|}}{C}\text{-}COOH$$

General procedure: (After Waters)

To a 500-ml., three-necked, round-bottomed flask equipped with a stirrer, condenser and two dropping funnels was added a suitable amount of sodium hydroxide, acetone and β-phenyl-α-oximinopropionic acid. The contents were slowly raised to reflux temperature while benzyl chloride and a solution of sodium hydroxide were added at approximately equal rates. After the addition was completed, the

mixture was refluxed for a definite period of time, after which the acetone was removed under reduced pressure, water being added as necessary to prevent the mixture from solidifying. The benzyloximino acid was then isolated, if possible using various procedures.

Waters extracted the alkaline mixture, after removing the acetone, with ether. At this point, it was observed that the sodium salt of the benzyloximino acid was also extracted along with the benzyl alcohol. The free acid was obtained by washing the benzyl alcohol from the sodium salt with cold absolute ether, after which the acid was obtained on acidification with hydrochloric acid.

a. Waters's Procedure.

Five-hundredths (.05) mole of oximino acid was dissolved in 25 ml. of 10% aqueous sodium hydroxide (.06 mole). After the addition of 100 ml. of acetone, .25 mole of benzyl chloride and 50 ml. of 34.7% aqueous sodium hydroxide (.26 mole) were added simultaneously. The mixture was refluxed two hours, after which the acetone was removed and the alkaline solution extracted with ether. The benzyloximino acid was isolated as mentioned above in 56% yield.

An investigation of this reaction was made to determine whether the five molar equivalents of benzyl chloride are necessary. In addition a study of the influence of temperature and alkali on the course of the reaction had to be carried out.

b. Influence of Alkali.

To .05 mole (9 gm.) of β-phenyl-α-oximinopropionic acid was added 50 ml. of 5% sodium hydroxide (.06 mole) and 25 ml. of acetone. While the temperature was slowly raised, .25 mole of benzyl chloride and 50 ml. of 35% sodium hydroxide (.44 mole) were added simultaneously. After refluxing one hour, the acetone was removed under reduced pressure, water being added to prevent the mixture from solidifying. At this point, the two layers present in the reaction flask were removed and allowed to separate. The lower alkaline layer, on acidification with dilute sulfuric acid, gave a white precipitate weighing 4 gm. and melting at 163-165°. Pure oximino acid melts at 165-169°. No β-phenyl-α-benzyloximinopropionic acid could be obtained from the organic layer after acidifying and extracting with ether. Undoubtedly, the excess alkali present hydrolysed the benzyl chloride before it reacted with the oxime. It will be shown later that too little alkali is also detrimental.

In almost all of the aqueous-acetone benzylations carried out in this work, acidification of the alkaline layer yielded some quantity of unchanged oxime. Waters did not attempt to work up his alkaline residues after extraction with ether. The fact that unchanged oxime may be isolated, however, is very important because it shows that the reaction under these conditions is not quantitative. It should be noted that one great difficulty in this

procedure is that the benzyl alcohol always comes over with the benzyloximino acid. Waters was able to remove the benzyl alcohol by cold ether extraction and probably lost some of the oxime ether in the process. Furthermore, crystallization of the α-benzyloximino acid from ethanol and water using decolorizing carbon invariably causes considerable loss of product.

c. Influence of temperature.

Instead of slowly raising the temperature while adding .25 mole of benzyl chloride and .24 mole of sodium hydroxide, the addition was carried out while the mixture was actively refluxing. After refluxing one hour and removing the acetone, the mixture (now neutral to litmus) was extracted with ether. The ether on evaporation yielded only a small quantity of white solid melting at 160°(unchanged oxime) and much dark residue, which was probably a mixture of benzyloximino acid and unchanged oxime. Only oils could be obtained, however, when recrystallization from acidified water and ethanol was attempted. Undoubtedly, the introduction of the benzyl chloride into the hot refluxing alkaline mixture is unsatisfactory, because of the undesirable condition of the product, which makes it impossible to recrystallize.

d. Reflux time.

As a rule, the mixtures were refluxed only one to two hours after the addition of the benzyl chloride and alkali, and never more than three hours. When the mixture was refluxed for six hours a black oil was obtained, from

which it was impossible to isolate any benzylated product.

e. Quantity of benzyl chloride.

No attempts were made to prepare the pure acid while varying the quantity of benzyl chloride. However, in a succeeding section (i), it will be shown that it is not necessary to use five equivalents of benzyl chloride, nor will one equivalent suffice. However, two equivalents of benzyl chloride give yields fully as satisfactory as five.

f. Removal of benzyl chloride by steam distillation.

It was felt that some better way of isolating the product, that is, freeing it from benzyl alcohol, was necessary to improve the process. With this in view, the following experiment was carried out, wherein the benzyl alcohol was removed by steam distillation.

To .05 mole of β-phenyl-α-oximinopropionic acid was added 50 ml. of 5% sodium hydroxide (.06 mole) and 25 ml. of acetone. Then .25 mole of benzyl chloride was added simultaneously with 26 ml. of 35% sodium hydroxide (.25 mole). After refluxing one-half hour, the benzyl alcohol was removed by distilling with steam for one hour. Ice was added, the mixture acidified with concentrated hydrochloric acid, extracted with ether and the ether extracted with 5% sodium hydroxide. When the alkaline extract was acidified with concentrated hydrochloric acid, an oil separated which was crystallized from ethanol and water. About 5 gm. (40% of theory) of product melting at 65° was obtained. The pure benzyloximino acid melts at 79°.

Recrystallization and decolorization with charcoal brought the melting point up to 78°.

This method of isolating the product is certainly easier than the other procedure used, but the extraction processes, although giving a better looking product, probably account for the lowered yields.

g. Benzylation with another agent.

It has been reported (81) that good yields of benzyl ethers of phenols could be obtained using benzylphenyldimethylammonium chloride as the benzylating agent. This was prepared by mixing dimethylaniline and benzyl chloride in chloroform and heating on the steam bath for eight hours, after which absolute ether was added to precipitate the light-gray solid product.

In a 500-ml., 3-necked, round-bottomed flask were placed 9 gm. (.05 mole) of β-phenyl-α-oximinopropionic acid and 80 ml. of 5% sodium hydroxide solution (.1 mole). To the refluxing solution was added 15 gm. (.06 mole) of benzylphenyldimethylammonium chloride in 25 ml. of water. After refluxing for three hours, the alkaline mixture was extracted with ether from which no product could be isolated. However, the aqueous portion when acidified and extracted with ether gave almost quantitatively on evaporation the original oxime. Under these conditions, the agent is not satisfactory for the benzylation of oximino acids.

h. Benzylation in alcohol.

In a 500-ml., 3-necked, round-bottomed flask equipped

with a stirrer and a condenser was placed 150 ml. of commercial absolute alcohol. This this was added 2.3 gm. (.1 mole) of sodium. After the sodium had reacted, 9 gm. (.05 mole) of finely powdered β-phenyl-α-oximinopropionic acid was added and then 12.6 gm. (.1 mole) of benzyl chloride was added to the hot solution. The mixture was refluxed for two hours and allowed to cool. Water and 75 ml. of 20% sodium hydroxide were added to the solid in the flask and the mixture heated on a hot plate for one-half hour. The mixture was extracted with chloroform and the aqueous layer acidified and extracted with ether. The chloroform extract on evaporation yielded 9.5 gm. of solid, presumably the sodium salt of the β-phenyl-α-benzyl-oximinopropionic acid, The ether extract yielded 3.5 gm. of material which was presumably the free acid. The dried residues were combined, dilute hydrochloric acid and ethanol were added and the mixture allowed to crystallize. Seven grams of white solid melting at 78-79° was obtained, representing a 52% yield.

This method was encouraging since no unreacted oxime was isolated. The following modification gave a better yield. The same quantities of reagents were used as above and after refluxing the mixture until it was neutral to litmus (2 to 4 hours) and cooling, the solid material was filtered off. The solid after drying was then dissolved in water and hydrochloric acid and extracted with ether. On evaporation of the ether 8.6 gm. of white solid, melting at 78-79° was obtained. This represents a 64% yield.

Even though this procedure gave good yields for this benzyloximino acid, it was felt that some benzyl ester might be lost in the alcohol. Furthermore, some of the oximes of lower molecular weight did not crystallize as well from the alcoholic reaction mixture. Consequently, the following procedure was adopted and has shown rather general application for the preparation of these benzyloximino acids.

<u>β-Phenyl-α-benzyloximinopropionic acid.</u>

$$C_6H_5-CH_2-\underset{\underset{NO-CH_2-C_6H_5}{\|}}{C}-COOH$$

In a 1-liter, 3-necked, round-bottomed flask equipped with a stirrer, condenser and drying tube were placed 500 ml. of commercial absolute alcohol and 11.5 gm. (.5 mole) of freshly cut sodium. After the sodium had reacted, 45 gm. (.25 mole) of finely divided β-phenyl-α-oximinopropionic acid was added. Then to the hot solution, 64 gm. (.5 mole) of benzyl chloride was added all at once and the mixture refluxed for two hours, or until it became neutral. Then 100 ml. of 20% potassium hydroxide in 95% ethanol was added and about 400 ml. of alcohol was distilled out of the mixture. Water and enough hydrochloric acid to make the solution acid were added and the mixture extracted with ether. The ether was removed in vacuo along with a good portion of the benzyl alcohol. The dry material in the flask was then dissolved in ethanol and water and

allowed to crystallize. Usually a quantitative yield of brown solid was obtained. When this material was recrystallized from ethanol-water and decolorized with Nuchar, the yield of solid was 43.5 gm. (66%), melting at 78-79°. Waters (38) reported 79-80°.

α-Benzyloximinopropionic acid.

$$CH_3-C(=NO-CH_2-C_6H_5)-COOH$$

Using the alcohol method, the solid obtained after reaction was dissolved in water, made alkaline, extracted with ether to remove some traces of benzyl alcohol, acidified with hydrochloric acid and extracted with ether. The ether on evaporation gave the desired product. Undoubtedly, some product was lost on the first extraction with ether. This procedure, using 25.8 gm. (.25 mole) of α-oximinopropionic acid gave 27.5 gm. (57%) of α-benzyloximinopropionic acid, a white solid melting at 73-75°, after recrystallization from ethanol and water.

α-Benzyloximinobutyric acid.

$$CH_3-CH_2-C(=NO-CH_2-C_6H_5)-COOH$$

A quantitative yield of crude product was obtained when the reaction mixture was treated with alcoholic potassium hydroxide and excess alcohol removed. However, some difficulty was encountered in purifying the product

from ligroin and isopropanol. It is felt that a better yield would have been obtained if the usual ethanol-water recrystallization had been used. From 20.7 gm. of oximino acid, 16.4 gm. of product was obtained (45% of theory), which melted at 86° when recrystallized from very dilute ethanol.

α-Benzyloximinoisocaproic acid.

$$CH_3-CH(CH_3)-CH_2-C(=NO-CH_2-C_6H_5)-COOH$$

Using the procedure as given for α-benzyloximinopropionic acid, a 68% yield of product was obtained. When the alcohol method incorporating alcoholic potassium hydroxide was used, a 79% yield was obtained. The white crystals, after recrystallization from ethanol and water, melted at 79-80°.

i. β-Phenyl- α-benzyloximinopropionyl chloride.

The aim of this problem was to prepare the acid chloride from the α-benzyloximino acid, so that the peptide linkage could be established when the acid chloride was reacted with amino acids. Since the α-benzyloximino acids proved to be difficult to isolate from the aqueous benzylation mixtures, this isolation was circumvented. The following procedure gave best results.

In a 1-liter, 3-necked, round-bottomed flask equipped with a stirrer, two dropping funnels and condenser were

placed 45 gm. (.25 mole) of β-phenyl-α-oximinopropionic acid, 125 ml. of 10% sodium hydroxide (.3 mole) and 250 ml. of acetone. While the temperature was slowly raised to reflux, 64 gm. (.5 mole) of benzyl chloride and 52 ml. of 35% sodium hydroxide (.45 mole) were added simultaneously over a period of one-half hour. The mixture was refluxed one and one-half hours and the acetone removed under reduced pressure. Benzene was added to the mixture and the lower aqueous layer separated. The benzene layer was washed with dilute hydrochloric acid and then dried over anhydrous magnesium sulfate. The dry, light-brown solution was filtered into a 1-liter, round-bottomed flask equipped with a condenser and drying tube. Then 90 gm. (.75 mole) of redistilled thionyl chloride was added and the mixture refluxed one hour, during which it usually turned quite dark. Another 30 gm. (.25 mole) of thionyl chloride was added and the refluxing continued another hour. The excess benzene and thionyl chloride were distilled off and the fraction boiling at 162-167° at .75-1.0 mm. pressure was collected. The yield of light-brown liquid varied from 46-49% of theoretical accounting for the oxime recovered. The material is colorless when pure.

When only one molar equivalent of benzyl chloride was used, no product was obtained. The yields actually seem to be better when two equivalents of benzyl chloride were used rather than five.

Unfortunately, this method for preparing the acid

chloride is fraught with one difficulty. When the distillation of the product us carried out, the distilling flask always contains a good portion of heavy black oil, part of which distils at a much higher temperature than the product. This oil tends to hold back the product, and at times, it was necessary to raise the temperature of the distilling bath to 250° to obtain the last portions of acid chloride. Attempts to work up the heavy oil were unsuccessful. The color of the residue may be due to the presence of some unreacted oximino acid, which is known to form tars with thionyl chloride.

III. ACID CHLORIDES

One method for the preparation of the acid chloride of β-phenyl-α-benzyloximinopropionic acid has been given in a previous section (II,i). However, the difficulties of this procedure and the color of the product often gave in turn poor amides when reacted with the amino acids. Consequently, it was felt advisable to prepare the acid chlorides from purer starting materials, which were made available in quantity through alkylation in ethanol.

Waters observed some difficulty in the preparation of the acid chlorides using thionyl chloride. The same difficulty was observed in the work repeated here. Furthermore, thionyl chloride, though redistilled, does not seem to carry the reaction to completion. In addition, it is

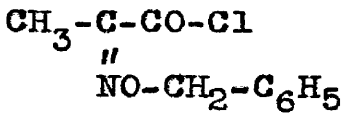
necessary to reflux the acid with thionyl chloride to obtain any product at all. Therefore, phosphorus pentachloride was used in place of thionyl chloride with better results. The reaction usually takes place at room temperature in benzene (quite vigorously at times) and never requires more than slight warming. The by-product of the reaction, phosphorus oxychloride, distils below the acid chlorides of the α-benzyloximino acids. Phosphorus pentachloride is a rather difficult chemical to weigh accurately, however, and its purity must be accepted at face value. It has a tendency to sublime, when unreacted, consequently coloring the acid chlorides slightly yellow. This never affects the quality of the amides, however.

It was observed that the use of phosphorus pentachloride and thionyl chloride together gave better yields than either alone. By using slightly less than the theoretical amount of phosphorus pentachloride and then adding thionyl chloride, the color of the product improved and no residues or sublimation was noted. The excess thionyl chloride is removed with the lower boiling fractions.

The benzyloximino acid chlorides are all colorless liquids when pure. β-Phenyl-α-benzyloximinopropionyl chloride has little odor, but the acid chlorides of lower molecular weight possess peculiar, pungent, straw-like odors. All of the acid chlorides boil under reduced pressures without appreciable decomposition. They slowly turn dark on storage. Waters showed that, even when stored

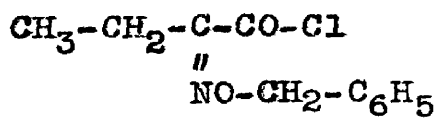
in ampules, these acid chlorides darkened after several months. When poured into water they do not generate heat, but seem to react only slowly. No attempt was made to analyse the acid chlorides, but they were all characterized through their anilides. Residues of the acid chlorides turned to a red solid when stored in glass-stoppered bottles for several months.

α-Benzyloximinopropionyl chloride.



α-Benzyloximinopropionic acid, 9.65 gm. (.05 mole), and 18 gm. (.15 mole) of thionyl chloride were refluxed in 100 ml. of benzene for three hours. The excess thionyl chloride and benzene were removed under reduced pressure and the liquid product was collected at 95-100°/.75 mm. The yield was 4.7 gm., 45% of the theoretical. The time of refluxing for this compound was too long; it is felt that phosphorus pentachloride would have given better yields.

α-Benzyloximinobutyryl chloride.



To 15.6 gm. (.075 mole) of α-benzyloximinobutyric acid dissolved in 100 ml. of benzene was added 15.6 gm. (.075 mole) of phosphorus pentachloride in two portions. Considerable heat was generated and some product was lost

in the foam of hydrogen chloride. Then 5 ml. of thionyl chloride was added and, after the benzene and excess thionyl chloride were removed, the product distilled. The yield of liquid boiling at 114°/1.5 mm. was 13.4 gm. (80% of theory).

α-Benzyloximinoisocaproyl chloride.

$$CH_3-CH(CH_3)-CH_2-\underset{\underset{NO-CH_2-C_6H_5}{\|}}{C}-CO-Cl$$

α-Benzyloximinoisocaproic acid, 11.75 gm. (.05 mole), and 24 gm. (.20 mole) of thionyl chloride were refluxed in 100 ml. of benzene for one hour. After removing the excess thionyl chloride and benzene, 8.3 gm. (65% of theoretical) of liquid boiling at 105-107°/.3 mm. was collected.

When the mixture was refluxed for two hours, the yield dropped to 40% of theory. A better yield was obtained using phosphorus pentachloride.

The α-benzyloximino acid, 23.5 gm. (.1 mole) was dissolved in 250 ml. of benzene and 20.8 gm. (.1 mole) of phosphorus pentachloride was added. After a short induction period, a rather violent reaction set in (hydrogen chloride evolved) and some product was lost through the condenser. When the product was distilled (117-118°/.85 mm.), 16.9 gm. (67% of theory) of liquid was obtained.

β-Phenyl- α-benzyloximinopropionyl chloride.

$$C_6H_5-CH_2-\underset{\underset{NO-CH_2-C_6H_5}{\|}}{C}-CO-Cl$$

To 26.9 gm. (.1 mole) of β-phenyl-α-benzyloximinopropionic acid in 200 ml. of benzene was added 20.8 gm. (.1 mole) of phosphorus pentachloride, in several portions, shaking the flask after each addition. The mixture was then warmed for one-half hour. On distillation, 20.8 gm. (73% of theoretical) of liquid boiling at 171°/.8 mm. was collected.

When the same quantities of benzyloximino acid and phosphorus pentachloride were used and few ml. of thionyl chloride were added, 24.1 gm. (84% of theory) of product boiling at 164°/.7 mm. was obtained.

IV. AMIDES AND ANILIDES

Coupling the amino acids with the alkoximino acid halides proved successful. This was accomplished using a modification of the method of McKie (82). The feature of this method is acylation in a two phase system of aqueous alkali and ether; that is, the acid chloride is added to the ether-alkali mixture containing the material to be acylated. Such a method saves valuable intermediates, in the case at hand the amino acids, and allows easy isolation of the reaction product. Furthermore, acid-sensitive substances such as tryptophane are easily acylated.

The α-benzyloximino acid anilides were not prepared by this method because of the availability of aniline to

serve as base to neutralize the hydrogen chloride liberated and of the ease of separation of aniline hydrochloride from the anilide produced. All of the anilides prepared in this work were white solids melting below 100°. They were sometimes difficult to crystallize, cooling and stirring being necessary to induce crystallization.

The α-benzyloximino acid amides of the amino acids were usually white solids melting around 100°, but in several cases difficulty was encountered in crystallization. Like the anilides, they required cooling in many cases and repeated stirring to induce crystallization. This sometimes was a barrier in purification of samples for analysis, since the products repeatedly separated as oils from the crystallizing medium. The amides of lower molecular weight were somewhat soluble in water, but the higher amides could be crystallized, as a rule, almost quantitatively from ethanol and water.

1. Derivatives of β-phenyl- α-benzyloximinopropionic acid.

β-Phenyl- α-benzyloximinopropionanilide.

$$C_6H_5-CH_2-\underset{\underset{NO-CH_2-C_6H_5}{\|}}{C}-CO-NH-C_6H_5$$

To 1.9 gm. (.02 mole) of aniline and 20 ml. of dry benzene in a small beaker was added, with continuous stirring, 2.9 gm. (.01 mole) of β-phenyl- α-benzyloximinopropionyl chloride. During the addition, a light-yellow

precipitate formed and the contents of the beaker became warm. The mixture was allowed to stand several hours, the aniline hydrochloride was filtered off and the benzene evaporated from the filtrate on the steam bath. The yellow oil remaining was taken up in ethanol and water and allowed to cool. An oil separated which after cooling crystallized. The crystals were filtered off and dried in vacuo over phosphorus pentoxide. The anilide melted at 72-73°; yield, 2.8 gm. (81% of theory).

Waters reported a melting point of 73.5-74°.

β-Phenyl-α-benzyloximinopropionyl glycine.

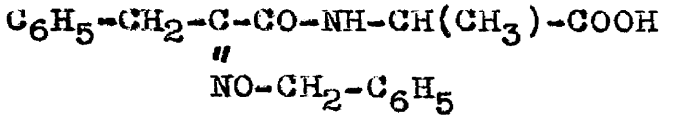
$$C_6H_5-CH_2-\underset{\underset{NO-CH_2-C_6H_5}{\|}}{C}-CO-NH-CH_2-COOH$$

Glycine, .75 gm. (.01 mole), was dissolved in 4 ml. of 10% sodium hydroxide (.01 mole) and 6 ml. of water in a small Erlenmeyer flask. The solution was overlayered with 10 ml. of ether. Then 2.9 gm. (.01 mole) of β-phenyl-α-benzyloximinopropionyl chloride in 10 ml. of absolute ether was added alternately, while stirring, with 4 ml. of 10% sodium hydroxide (.01 mole) in 6 ml. of water. A little heat was developed in the reaction flask. The mixture was then allowed to stand in the refrigerator overnight. The lower alkaline layer was neutralized with a small amount of concentrated hydrochloric acid. The oil which separated crystallized, after being cooled. After

drying in vacuo over phosphorus pentoxide, 2.95 gm. (90% of theory) of white solid was obtained. After recrystallization from ethanol and water, the product melted at 96.5-97°.

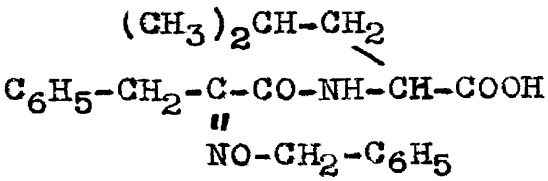
In larger runs, the yields ranged up to 98%. This procedure exemplifies the method for the preparation of these amides.

β-Phenyl- α -benzyloximinopropionylalanine.



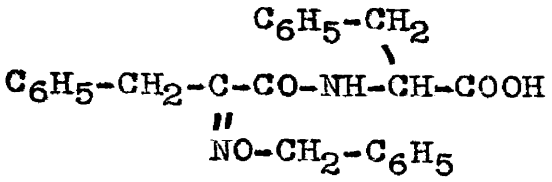
Using the general procedure given above, 2.9 gm. (.01 mole) of acid chloride and .89 gm. (.01 mole) of alanine gave 3.08 gm. (91% of theory) of white solid, which melted, after recrystallization from dilute alcohol at 112°.

β-Phenyl- α - benzyloximinopropionyl-l(-)leucine.



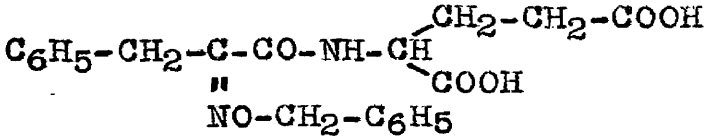
Using the method outlined, 2.9 gm. (.01 mole) of acid chloride and 1.31 gm. (.01 mole) of l(-)leucine gave 2.93 gm. (77% of theory) of white solid, which melted at 86-87° on recrystallization from aqueous alcohol.

β-Phenyl-α-benzyloximinopropionyl-β-phenylalanine.



When the higher amino acids, such as phenylalanine, were used a somewhat larger quantity of water was necessary for initial solution. Otherwise the procedures are exactly the same. From 2.9 gm. (.01 mole) of acid chloride and 1.65 gm. (.01 mole) of phenylalanine was obtained 3.51 gm. (85% of theory) of white solid, which melted at 140-141° after recrystallization from ethanol and water. This amide did not separate as an oil, but always separated as a solid when the hydrochloric acid was added to the alkaline mixture.

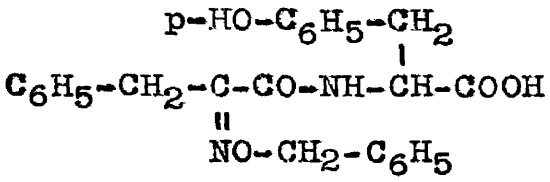
β-Phenyl-α-benzyloximinopropionyl-l(+)glutamic acid.



l(+)Glutamic acid, 2.94 gm. (.02 mole) was dissolved in 4.8 ml. of 32.5% sodium hydroxide (.04 mole) and 20 ml. of water. To this solution was added 20 ml. of ether. Then 5.76 gm. (.02 mole) of β-phenyl-α-benzyloximinopropionyl chloride in 20 ml. of absolute ether was added alternately, while shaking, with 2.4 ml. of 32.5% sodium hydroxide (.02 mole) in 15 ml. of water. The mixture was allowed to stand in the refrigerator overnight, the lower alkaline layer was separated and acidified with

hydrochloric acid. In this manner, 3.3 gm. of product was isolated. On cencentration of the mother liquor, another 1.3 gm. was obtained. The overall yield of product melting at 110° was 58%.

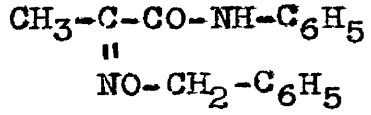
β-Phenyl-α-benzyloximinopropionyl-l(-)tyrosine.



This product was very difficult to isolate and repeatedly came down as an oil. It was decolorized with charcoal and crystallized several times. Ultimately, fine white platelets melting at 67° were obtained. The yield was poor because of loss during repeated recrystallization. It is possible that the hydroxyl group was esterified.

2. Derivatives of α-benzyloximinopropionic acid.

α-Benzyloximinopropionanilide.



This anilide was prepared by the method described for β-phenyl-α-benzyloximinopropionanilide. From 2.1 gm. (.01 mole) of α-benzyloximinopropionyl chloride and 1.9 gm. (.02 mole) of aniline was obtained 2.25 gm. (84% of theory) of white needles melting at 70-71°.

α-Benzyloximinopropionylglycine.

$$CH_3-C(=NO-CH_2-C_6H_5)-CO-NH-CH_2-COOH$$

Using the general method described for the preparation of these amides, 1.06 gm. (.005 mole) of α-benzyloximinopropionyl chloride and .38 gm. (.005 mole) of glycine gave .74 gm. (60% of theory) of white solid melting at 127° when recrystallized from water.

α-Benzyloximinopropionylalanine.

$$CH_3-C(=NO-CH_2-C_6H_5)-CO-NH-CH(CH_3)-COOH$$

Using the general procedure, 1.06 gm. (.005 mole) of α-benzyloximinopropionyl chloride and .45 gm. (.005 mole) of alanine gave .71 gm. (54% of theory) of white solid melting at 118°, when recrystallized from 10% ethanol.

3. Derivatives of α-benzyloximinoisocaproic acid.

α-Benzyloximinoisocaproanilide.

$$CH_3-CH(CH_3)-CH_2-C(=NO-CH_2-C_6H_5)-CO-NH-C_6H_5$$

When 1.26 gm. (.005 mole) of α-benzyloximinoisocaproyl chloride and .93 gm. (.01 mole) of aniline were reacted as described for β-phenyl- α-benzyloximinopropionanilide,

1.18 gm. (77% of theoretical) of white solid was obtained, which on recrystallization from dilute alcohol, melted at 57°.

α-Benzyloximinoisocaproylglycine.

$$CH_3-CH(CH_3)-CH_2-C(=NO-CH_2-C_6H_5)-CO-NH-CH_2-COOH$$

When 1.26 gm. (.005 mole) of α-benzyloximinoisocaproyl chloride and .38 gm. (.005 mole) of glycine were reacted in the usual way, .65 gm. (45% of theory) of white solid was obtained, which melted at 58-59° on recrystallization from 10% alcohol. This series of amides was characterized by being unusually difficult to crystallize.

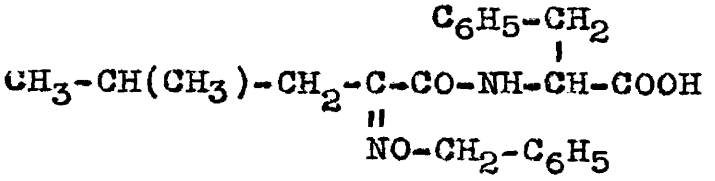
α-Benzyloximinoisocaproylalanine.

$$CH_3-CH(CH_3)-CH_2-C(=NO-CH_2-C_6H_5)-CO-NH-CH(CH_3)-COOH$$

Using the general procedure, 1.26 gm. (.005 mole) of α-benzyloximinoisocaproyl chloride and .45 gm. (.005 mole) of alanine gave .80 gm. (52% of theory) of white solid melting at 70° on recrystallization from dilute alcohol. The lowered yields of the last four amides was due to the increased solubility of the amides in water. In a repeat experiment, the mother liquor was concentrated, and 3.79 gm. (.015 mole) of acid chloride and 1.34 gm.

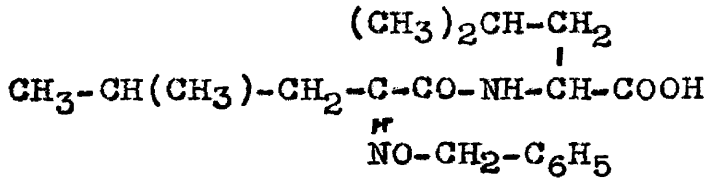
(.015 mole) of alanine gave 4.3 gm. of solid, representing a 94% yield.

α-Benzyloximinoisocaproyl-β-phenylalanine.



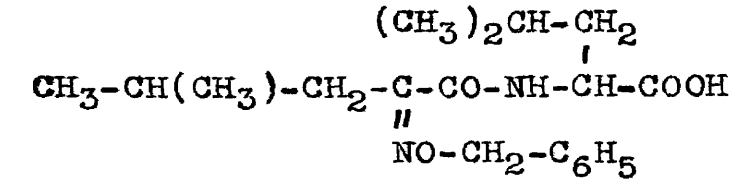
The general procedure, using 1.26 gm. (.005 mole) of α-benzyloximinoisocaproyl chloride and .825 gm. (.005 mole) of phenylalanine gave 1.37 gm. (73% of theory) of white microcrystals which melted at 116-116.5° on recrystallization from ethanol and water.

α-Benzyloximinoisocaproyl-l(-)leucine.



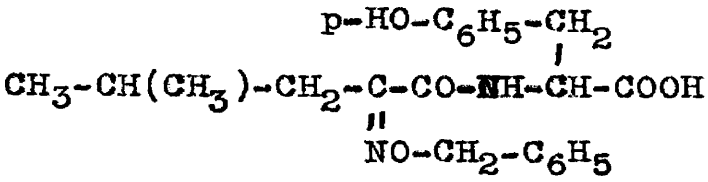
When 2.31 gm. (.017 mole) of l(-)leucine and 5.1 gm. (.02 mole) of acid chloride were reacted in the usual way, an oil separated from the acidulated water. All attempts to make this oil crystallize were fruitless. The difficulty was probably due to contamination with the acid.

α-Benzyloximinoisocaproyl-dl-leucine.



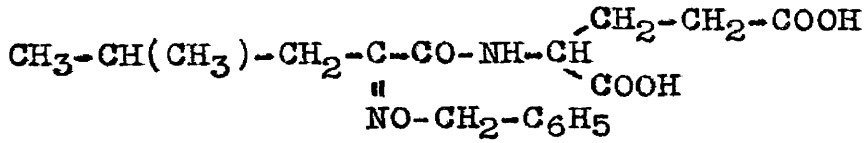
When 1.97 gm. (.015 mole) of dl-leucine and 3.79 gm. (.015 mole) of acid chloride were reacted in the usual manner, 4.8 gm. (92% of theory) of white solid was obtained, which melted at 45-46°, after crystallization from water and alcohol.

<u>α-Benzyloximinoisocaproyl-l(-)tyrosine</u>.



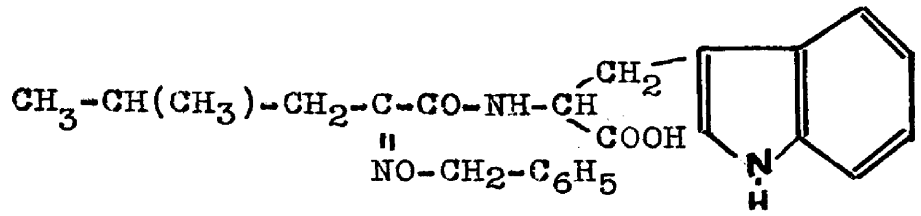
Using the general procedure, 2.72 gm. (.015 mole) of tyrosine and 3.79 gm. (.015 mole) of acid chloride gave 2.9 gm. of light-yellow solid melting at 78.5-79°. Yield, 46% of theory. The low analysis (page 72) indicates that the desired compound was not obtained.

<u>α-Benzyloximinoisocaproyl-l(+)glutamic acid</u>.



When 2.21 gm. (.015 mole) of l(+)glutamic acid and 3.79 gm. (.015 mole) of acid chloride were reacted in the usual manner, 4.3 gm. (74% of theory) of white solid melting at 101° was obtained.

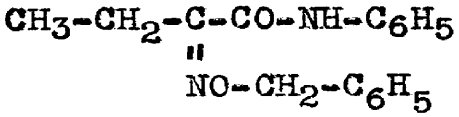
α-Benzyloximinoisocaproyl-l(-)tryptophane.



When 1.02 gm. (.005 mole) of l(-)tryptophane and 1.27 gm. (.005 mole) of acid chloride were reacted in the usual way, taking care to avoid excess acid, 1.8 gm. (86% of theory) of white solid melting at 90° was obtained. The product was recrystallized from ethanol and water for analysis.

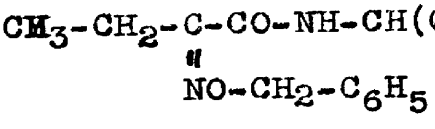
4. Derivatives of α-benzyloximinobutyric acid.

α-Benzyloximinobutyranilide.



When .57 gm. (.0025 mole) of α-benzyloximinobutyryl-chloride and .47 gm. (.005 mole) of aniline were reacted as described for β-phenyl- α -benzyloximinopropionanilide, .61 gm. (84% of theory) of white solid melting at 71° was isolated.

α-Benzyloximinobutyrylalanine.

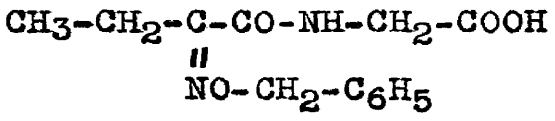


Using the general procedure for these amides as

described for β-phenyl- α-benzyloximinopropionylglycine, 4.56 gm. (.02 mole) of α-benzyloximinobutyryl chloride and 1.78 gm. (.02 mole) of alanine gave 4.73 gm. (84% of theoretical) of white crystals, which melted at 94° when recrystallized from ethanol and water.

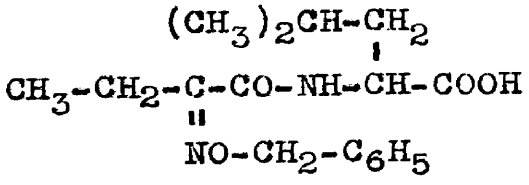
The amides obtained from α-benzyloximinobutyryl chloride and the amino acids were characteristic in that they rarely separated from the crystallizing medium as oils.

α-Benzyloximinobutyrylglycine.



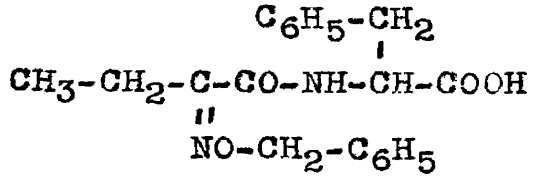
When 1.14 gm. (.005 mole) of α-benzyloximinobutyryl chloride and .38 gm. (.005 mole) of glycine were reacted in the usual manner, 1.20 gm. (90% of theoretical) of white crystals were isolated, which melted at 106° when recrystallized from dilute ethanol.

α-Benzyloximinobutyryl-dl-leucine.



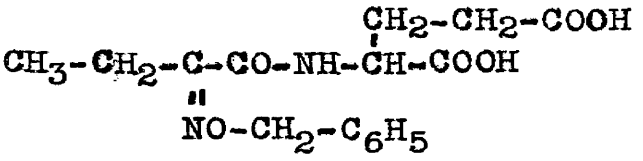
When 1.14 gm. (.005 mole) of acid chloride and .66 gm. (.005 mole) of dl-leucine were reacted according to the general procedure, 1.25 gm. (77% of theory) of white solid was isolated, which melted at 87° when recrystallized from alcohol and water.

α-Benzyloximinobutyryl-β-phenylalanine.



Using the general procedure, .83 gm. (.005 mole) of phenylalanine and 1.14 gm. (.005 mole) of acid chloride gave 1.46 gm. (82% of theory) of white needles, which melted at 89° after recrystallization from ethanol-water.

α-Benzyloximinobutyryl-l(+)glutamic acid.



The general procedure, using .82 gm. (.005 mole) of l(+)glutamic acid and 1.14 gm. (.005 mole) of acid chloride gave .93 gm. (52% of theory) of white solid. This material was exceptionally soluble in water and some product undoubtedly was lost in the acidulated water. Using the smallest amount of water and ethanol possible, a white product melting at 92-93° was obtained.

V. HYDROGENATIONS

Since the amides from the amino acids and the α-benzyloximino acid chlorides were so readily available, the next logical step was their reduction to the corresponding dipeptides. The catalytic debenzylation of certain

$$R\text{-}\underset{\underset{NO\text{-}CH_2\text{-}C_6H_5}{\|}}{C}\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOH \longrightarrow R\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOH$$

benzyl ethers has been discussed and the situation here seemed to be similar. Likewise, Hamlin's (31) success in reducing oximino acids to the corresponding amino acids encouraged the thought that the dipeptides should be readily available.

The debenzylation of the benzyloximino acid amides with certain catalysts has been established as positive. Both palladium and a mixture of palladium and platinum seem to accomplish this, either in ethanol, ethanolic hydrochloric acid or glacial acetic acid. The aqueous methanol-acetic acid and palladium catalyst method so elegantly utilized by Bergmann and Zervas (34) did not serve to debenzylate the benzyloxime ethers studied here.

The difficult part of the reduction to the free amino group lies in reducing the oxime, which is supposedly produced on the debenzylation of the ether, by the first mole of hydrogen, as follows:

$$R\text{-}\underset{\underset{NOCH_2\text{-}C_6H_5}{\|}}{C}\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOH \xrightarrow{1\ H_2} R\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOH$$

$$\xrightarrow{2\ H_2} \quad R\text{-}CH(NH_2)\text{-}CO\text{-}NH\text{-}CHR'\text{-}COOH$$

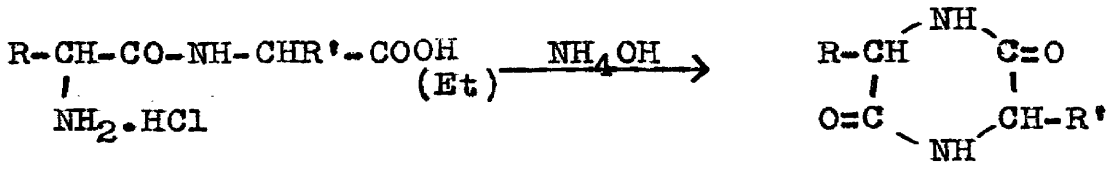
When the reduction is carried out in the presence of hydrochloric acid, the peptide hydrochloride should be produced. Ordinarily, this would not be thought to be a barrier, since Hamlin very successfully isolated the free amino acids by first evaporating the alcohol from the reduction mixture, dissolving the hydrochloride in boiling water and neutralizing with ammonium hydroxide. Addition of alcohol then precipitated the free amino acid. The situation as regards the solubility of the peptides in alcohol and water is largely parallel to that with the amino acids. Both have fair solubility in water depending on the molecular weight and both are largely insoluble in alcohol.

Consequently, when the theoretical amount of hydrogen was taken up on the reduction of the benzyloximino acid amides, a very similar procedure was followed. However, the substances isolated did not seem to be as soluble in water as was expected of the dipeptides. Nevertheless, the melting points checked the literature fairly closely. A closer investigation of this situation revealed some rather interesting facts, however. The diketopiperazines corresponding to the various dipeptides have melting points which are very close to the dipeptides themselves. The following chart shows the melting points of some dipeptides of phenylalanine and leucine and their corresponding diketopiperazines (anhydrides).

Peptide	Diketopiperazine
1. Phenylalanylalanine, 241°d. (83)	1. 267-268°d. (84)
2. Phenylalanylglycine, 273° (83)	2. 280° (85)
3. Phenylalanylphenylalanine 288° (86)	3. 290-291° (87)
4. Leucylalanine, 248° (88)	4. 247°

Since the melting points are relatively close at this high range, the melting point is of little value in differentiating between the dipeptide and anhydride. Moreover, both groups of compounds discolor before they reach their melting points.

The only immediate method for differentiation is solubility in acids and bases. Many of the compounds prepared in this work were not soluble in acids or bases, confirming that the peptide anhydrides were formed in the isolation or reduction.



Diketopiperazines are not ordinarily formed under conditions so mild as those used in this work. There are several methods by which symmetrical anhydrides are formed from the amino acids or their esters. Apparently, the method most used for preparation of the anhydrides from the peptides involves the use of the ethyl ester of the peptide in alcoholic ammonia (10, 11). Free peptides

ordinarily do not pass into anhydrides except when heated to rather high temperatures.

There are some indications also that the free peptides were obtained, but yields were low.

Palladium Catalyst for Reduction.

For the study of the reduction of benzyloximino acid amides, β-phenyl-α-benzyloximinopropionylalanine was used. The reductions, for the most part, were carried out at room temperature and 20 atmospheres pressure in a glass-lined vessel of such capacity that .01 mole of hydrogen gave approximately a 10-12 pound pressure drop, depending on the slight variations in amount of solvent used in the hydrogenations. The palladium catalyst was prepared by the method of Hartung (39).

1. β-Phenyl-α-benzyloximinopropionylalanine, 5.1 gm. (.015 mole), was dissolved in 100 ml. of ethanol, and 50 ml. of water and 25 ml. of glacial acetic acid were added. Then 5 gm. of 10% palladium catalyst on Nuchar WA was added and the mixture shaken at room temperature under 20 atmospheres of hydrogen. No hydrogen uptake was observed; the amide was recovered unreacted.

This procedure was repeated using 95% ethanol and a few ml. of concentrated hydrochloric acid. The vessel was heated to 70° and shaken. Again, no uptake of hydrogen was observed.

2. Fifteen-hundredths mole of amide was dissolved in 100 ml.

of 95% ethanol and 5 ml. of concentrated hydrochloric acid. After adding 5 gm. of 10% palladium catalyst, another .5 gm. of palladium chloride was added and the mixture was shaken at room temperature under 20 atmospheres of hydrogen. When the vessel was opened, a definite odor of toluene was noted. The catalyst was filtered off and the filtrate neutralized with aqueous sodium hydroxide and the filtrate evaporated on the steam bath. The residue was taken up in water and alcohol was added. A very small quantity of material melting at 268-270° precipitated. The diketopiperazine melts at 267-268° (84).

Calcd. for phenylalanylalanine anhydride; $C_{12}H_{14}N_2O_4$:
nitrogen, 12.84%; carbon, 66.03%; hydrogen, 6.47%.
Found:
nitrogen, 12.86%; carbon, 65.78%; hydrogen, 6.53%.

It was evident that while the hydrogen uptake was good, that it was improper to use sodium hydroxide in the neutralization.

After reductions of this type, the catalyst was treated with hot water and sometimes ammonium hydroxide. On evaporation of the water, no product was obtained. It was apparent that the reduction product was soluble in alcohol and hydrochloric acid. It was also interesting to note that after neutralization of the filtrate with ammonium hydroxide, that silver nitrate, when added to the mixture,gave a precipitate which continued to form even though the chloride ions were all theoretically

removed. This precipitate quickly discolored. The unreacted amide and amino acids do not form precipitates with silver nitrate under these conditions. Consequently, the material present might be the anhydride, since the theoretical amount of hydrogen was taken up.

An attempt was made to obtain β-phenyl- α-oximino-propionylalanine by hydrogenating the benzyl ether in alcohol and interrupting the hydrogenation when just enough hydrogen had been taken up to debenzylate the ether (1 mole). The filtrate was evaporated in a desiccator containing sulfuric acid and soda lime. An alkali soluble oil was obtained, which could not be crystallized.

Since a 10% catalyst failed to reduce and a fortified catalyst carried the reaction on, a 40% palladium catalyst was prepared and used in the reduction. At the end of three and one-half hours, the theoretical amount of hydrogen was taken up. The speed of the reduction was typical of those carried out in later studies with mixed palladium-platinum catalysts. The time for the three mole uptake was as follows:

first mole ---- 7 minutes - debenzylation
second mole -- 27 minutes
third mole -- 177 minutes - complete
Total 3 hrs. 31 minutes

When the hydrogenation of 3.4 gm. (.01 mole) of β-phenyl- α -benzyloximinpropionylalanine, in 95 ml. of ethanol and 5 ml. of hydrochloric acid with 5 gm. of 40% palladium catalyst, was complete; the filtrate was dried in vacuo and ammonium hydroxide and water added until the solution was neutral. The solution was concentrated and

alcohol added; 1.02 gm. of white solid separated on cooling. Some of this material was very soluble in water, indicating the presence of ammonium chloride. In all probability, the major part of the product was the diketopiperazine since all the solid was not soluble in sodium hydroxide or hydrochloric acid.

Reductions in ethanol without added hydrochloric acid would not take up the theoretical amount of hydrogen. When hydrochloric acid was added, the hydrogenations proceeded to completion.

3. Since the dipeptide esters are known to form diketopiperazines easily, it was thought that reductions in another medium would be more satisfactory. Using both a 10% and 40% palladium catalyst in glacial acetic acid, about two of the three moles of hydrogen were taken up. The acetic acid was removed under reduced pressure and the solid taken up in ethanol and neutralized with ammonium hydroxide. No precipitate occurred indicating presence of alcohol soluble material. Since the dipeptides are insoluble in ethanol, the dipeptide obviously was not formed. Concentration and addition of ether yielded only ammonium acetate.

Raney Nickel

Five and one-tenth gm. (.015 mole) of amide was dissolved in 100 ml. of 95% ethanol and .8 to 1.0 gm. of Raney nickel catalyst added. The mixture was shaken at

room temperature and 20 atmospheres with no observable uptake of hydrogen. The pressure was then boosted to 96 atmospheres and the mixture was shaken again. Still no significant drop in hydrogen occurred. The vessel was then heated to 75° and rocked for two hours. On cooling, the uptake of hydrogen seemed to be quantitative or nearly so, although a three atmosphere drop at pressures such as these was difficult to detect. The alcohol had no definite odor of toluene, but on evaporation yielded an oil, insoluble in water. There was isolated a small portion of unreacted amide. The oil was insoluble in alkali and soluble in hydrochloric acid. However, when dissolved in ether-benzene, dried over anhydrous magnesium sulfate and treated with dry hydrogen chloride, no precipitate occurred. The course of this reaction was uncertain.

Mixed Palladium-platinum Catalyst.

A number of reductions using Hartung's palladium catalyst with added Adams' platinum catalyst (89) were carried out on β-phenyl- α-benzyloximinopropionylalanine. 1. When .015 mole of amide was reduced with 5 gm. of 10% palladium catalyst with .15 gm. of added platinum in 95 ml. of ethanol and 5 ml. of concentrated hydrochloric acid, the theoretical amount of hydrogen was taken up in three and one-half hours. The ethanol was removed in vacuo and ammonium hydroxide added. The material was dried on the steam bath and extracted with absolute alcohol.

A precipitate weighing .4 gm. separated from the alcohol and melted at 240°. The dipeptide melts at 241° (83).

Calcd. for $C_{12}H_{16}N_2O_3$: nitrogen, 11.86%; carbon, 61.00%; hydrogen, 6.84%. Found: nitrogen, 11.38%; carbon, 62.98%; hydrogen, 7.48%.

The analysis indicates some impurity. The residue also yielded .32 gm. of a water and alcohol insoluble product melting at 254-258° with much decomposition. The analyses indicated that ammonium chloride with some organic material was present, presumably the diketopiperazine.

Another reduction using the same procedure was carried out. Hot water was added to the residue which had been treated with ammonium hydroxide. Some sticky product remained behind, but a solid separated from the water, melting at 246-254°d. Analysis indicated the diketopiperazine contaminated, perhaps, with some dipeptide.

Calcd. for $C_{12}H_{14}N_2O_2$: nitrogen, 12.84%; carbon, 66.03%; hydrogen, 6.47%. Found; nitrogen, 13.05%; carbon, 64.62%; hydrogen, 7.01%.

The following procedure gave fair yields of the diketopiperazine. When 5.1 gm. (.015 mole) of amide was reduced with 5 gm. of 10% palladium catalyst with .15 gm. of added platinum oxide in 95% ethanol and hydrochloric acid, the theoretical amount of hydrogen was taken up. The filtrate was treated with 10 ml. of concentrated ammonium hydroxide and evaporated in vacuo. About 300 ml.

of water was added to the residue (wt., 6 gm.). Some of the material did not dissolve, .76 gm., but .73 gm. separated from the water on cooling. The material was insoluble in acid and alkali, and melted at 248-252°. Most of the material that analysed for diketopiperazine in this work melted somewhat below the reported value.

When 4.89 gm. (.015 mole) of β-phenyl-α-benzyl-oximinopropionylglycine was reduced under these conditions, a solid and an alcohol soluble oil remained. The solid was treated with alcohol and water and on filtering, 1.3 gm. of solid melting at 263-268°d. was isolated. This white product was insoluble in acid and alkali and is presumably the anhydride although it melts more than 10° below the melting point of the inactive anhydride. It is interesting to note that the active laevo form of the anhydride melts at 265.5°d. (90).

When 4.16 gm. (.01 mole) of β-phenyl-α-benzyloximinopropionylphenylalanine was reduced under the same conditions, .5 gm. of solid melting at 283° was obtained. Its solubility in alkali and acid indicated that the diketopiperazine was formed.

In all these reductions, there is apparently considerable alcohol soluble material formed which is strange since the anhydrides and dipeptides are insoluble in alcohol.

Even though the theoretical amount of hydrogen was taken up, apparently some other products are formed in the reaction. It is important to note that the ethyl esters of the peptides are much more soluble in alcohol than the free peptides.

2. When β-phenyl-α-benzyloximinopropionylalanine was reduced in ethanolic hydrochloric acid with added water, a small quantity of substance (8%) melting at 240°d. was obtained. This material was soluble in alkali and acid and is probably the free peptide. Addition of water seems to favor free peptide formation.

3. Reductions in glacial acetic acid with palladium-platinum catalysts didn't take up the theoretical amount of hydrogen. Removal of the acetic acid in vacuo gave alcohol soluble products and certainly not the diketopiperazine or peptide.

Indications are that the benzyl group is rather readily removed by catalytic hydrogenation. However, the subsequent course of the reduction and the influence of solvent is obscure. Ethanol may form the peptide ester which would account for the ready formation of anhydrides and alcohol soluble substances found in the reductions. The addition of water to the ethanol certainly cuts down anhydride formation and seems to help peptide formation, although the yields are low. The encouraging factor is that the benzyl ethers are readily split under rather easy conditions and would indicate that the selection of a good

reducing medium would solve the problem of ester formation (if this happens) and subsequent anhydride formation.

The difficulty is apparently not limited to the phenylalanine derivatives. When α-benzyloximinoisocaproylalanine was reduced in aqueous ethanol with a platinum-palladium catalyst, some small amount of solid melting at 245-250° was isolated. However, this material was rather soluble in alcohol. With the diketopiperazines of lower molecular weight, the alcohol solubility is much greater than with the phenylalanyl derivatives. The lower peptides, such as leucylalanine, however, are not very soluble in alcohol.

VI. INCREASING PEPTIDE CHAIN

If it were possible to increase the number of amino acids in the chain, the probability of diketopiperazine formation would be eliminated. Furthermore, if the reduction to the dipeptide itself is accomplished in good yield, an extension of the chain would be most desirable. Therefore, several experiments were carried out on β-phenyl-α-benzyloximinopropionylalanine with this in mind.

The benzyloximino acid amide, 1.7 gm. (.005 mole), was dissolved in 100 ml. of benzene and .02 mole of thionyl chloride was added. The solution was refluxed for one hour and the excess thionyl chloride and benzene

were removed under reduced pressure. The crude material remaining was reacted with .005 mole of aniline according to the general procedure for making amides of the amino acids. The lower alkaline layer was extracted with benzene and neutralized with hydrochloric acid. A dark gum separated which could not be crystallized.

Phosphorus pentachloride was used instead of thionyl chloride. When the amide in decalin was refluxed with phosphorus pentachloride, the reaction turned red and a black material separated. Consequently, the phosphorus pentachloride when refluxed at this high temperature is deleterious.

Therefore the reaction was carried out at room temperature. One-hundredth mole of amide was dissolved in 50 ml. of absolute ether and .01 mole of phosphorus pentachloride was added. The reaction mixture was agitated and after thirty minutes was added to .02 mole of aniline in 25 ml. of absolute ether. The white precipitate was filtered off and washed with water to remove the aniline hydrochloride. The residue remaining was crystallized from ethanol and water and dried over phosphorus pentoxide. The white solid, .3 gm., melted at 102°. The ether filtrate on evaporation yield a red product which was very difficult to decolorize with charcoal. Indications are that the solid isolated was the anilide of β-phenyl-α-benzyloximinopropionylalanine.

This would indicate that the extension of the peptide chain is possible.

SUMMARY

I. A study of the benzylation of oximino acids has been carried out and the yields improved over the existing method. The influence of reaction temperature, time of refluxing and ratio of reagents on the course of the alkylation in aqueous acetone has been studied. It is shown that alkylation in alcohol is more satisfactory than alkylation in aqueous acetone. Several new α-benzyloximino acids have been prepared.

II. The preparation of the α-benzyloximino acid chlorides has been carried out in good yield and the superiority of the phosphorus pentachloride method over thionyl chloride, alone, is demonstrated.

III. It has been shown that these acid chlorides may be coupled with a variety of amino acids, usually in good yield. A number of these new amides have been prepared.

IV. A study of the reduction of these amides has shown that the benzyl group is readily removed by palladium and platinum catalysts at room temperature and at low pressures. The reaction products isolated indicate that the oxime ethers are reduced to the free amines in ethanol and aqueous ethanol with added hydrochloric acid. The isolation of diketopiperazines from these reduction mixtures suggests that the ethyl ester is formed in ethanol and hydrochloric acid, or that an exceptionally easy

reaction for the formation of diketopiperazines has been uncovered.

V. Preliminary indications show that the acid chlorides of the amides of the amino acids and benzyloximino acid chlorides can be prepared, so that the synthesis can be extended to peptides containing more than two amino acid residues.

TABLE I

NEW COMPOUNDS PREPARED

Name	Page	Properties
1. α-Benzyloximino-propionic acid	36	m. p. 73-75°. Nitrogen: Calcd. : 7.26% Found : 7.07%
2. α-Benzyloximino-butyric acid	36	m. p. 86°. Nitrogen: Calcd. : 6.79% Found : 6.92%
3. α-Benzyloximino-isocaproic acid	37	m. p. 79-80°. Nitrogen: Calcd. : 5.95% Found : 6.23%
4. α-Benzyloximino-propionyl chloride	41	b. p. 95-100°/.75 mm.
5. α-Benzyloximino-butyryl chloride	41	b. p. 114°/1.5 mm.
6. α-Benzyloximino-isocaproyl chloride	42	b. p. 105-107°/.3 mm.
7. β-Phenyl-α-benzyl-oximinopropionyl-glycine	45	m. p. 73.5°. Nitrogen: Calcd. : 8.59% Found : 8.70%
8. β-Phenyl-α-benzyl-oximinopropionyl-l(-)leucine	46	m. p. 86-87°. Nitrogen: Calcd. : 7.33% Found : 7.10%
9. β-Phenyl-α-benzyl-oximinopropionyl-alanine	46	m. p. 112°. Nitrogen: Calcd. : 8.23% Found : 8.46%
10. β-Phenyl-α-benzyl-oximinopropionyl-phenylalanine	47	m. p. 140-141°. Nitrogen: Calcd. : 6.73% Found : 6.97%
11. β-Phenyl-α-benzyl-oximinopropionyl-l(+)glutamic acid	47	m. p. 110°. Nitrogen: Calcd. : 7.03% Found : 6.90%
12. β-Phenyl-α-benzyl-oximinopropionyl-l(-)tyrosine	48	m. p. 67°. Nitrogen: Calcd. : 6.48% Found : 5.76% Calcd. for bis comp. : 6.15%

Micro-analyses by Oakwold Laboratories, Alexandria, Va.

TABLE I (Continued)

Name	Page	Properties
13. α-Benzyloximino-propionanilide	48	m. p. 70-71°. Nitrogen: Calcd. : 10.44% Found : 10.75%
14. α-Benzyloximino-propionylglycine	49	m. p. 127°. Nitrogen: Calcd. : 11.20% Found : 11.39%
15. α-Benzyloximino-propionylalanine	49	m. p. 118°. Nitrogen: Calcd. : 10.14% Found : 10.43%
16. α-Benzyloximino-isocaproanilide	49	m. p. 57°. Nitrogen: Calcd. : 9.03% Found : 9.15%
17. α-Benzyloximino-isocaproylglycine	50	m. p. 58-59°. Nitrogen: Calcd. : 9.56% Found : 8.07%
18. α-Benzyloximino-isocaproylalanine	50	m. p. 70°. Nitrogen: Calcd. : 9.15% Found : 8.81%
19. α-Benzyloximino-isocaproylphenyl-alanine	51	m. p. 116-116.5°. Nitrogen Calcd. : 7.33% Found : 7.33%
20. α-Benzyloximino-isocaproyl-dl-leucine	51	m. p. 45-46°. Nitrogen: Calcd. : 8.09% Found : 8.25%
21. α-Benzyloximino-isocaproyl-l(-)tyrosine	52	m. p. 78.5°-79°. Nitrogen: Calcd. : 7.01% Found : 6.06% Calcd. for bis comp.: 6.82%
22. α-Benzyloximino-isocaproyl-l(+)glutamic acid	52	m. p. 101°. Nitrogen: Calcd. : 7.69% Found : 7.41%
23. α-Benzyloximino-isocaproyl-l(-)trypto-phane	53	m. p. 90°. Nitrogen: Calcd. : 9.97% Found : 10.72%
24. α-Benzyloximino-butyranilide	53	m. p. 71°. Nitrogen: Calcd. : 9.93% Found :10.33%

TABLE I (Continued)

Name	Page	Properties
25. α-Benzyloximino-butyrylalanine	53	m. p. 94°. Nitrogen: Calcd. : 10.06% Found : 10.31%
26. α-Benzyloximino-butyrylglycine	54	m. p. 106°. Nitrogen: Calcd. : 10.60% Found : 9.86%
27. α-Benzyloximino-butyryl-dl-leucine	54	m. p. 87°. Nitrogen: Calcd. : 8.75% Found : 8.39%
28. α-Benzyloximino-butyrylphenylalanine	55	m. p. 89°. Nitrogen: Calcd. : 7.91% Found : 8.17%
29. α-Benzyloximino-butyryl-l(+)glutamic acid	55	m. p. 92-93°. Nitrogen: Calcd. : 8.33% Found : 7.89%
30. β-Phenyl- α -benzyl-oximinopropionylalanyl-anilide	68	m. p. 102°. : Nitrogen: Calcd. : 10.11% Found : 9.67%

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